CHEMISTRY OF *Food and Nutrition*

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CHEMISTRY OF
Food and Nutrition

BY

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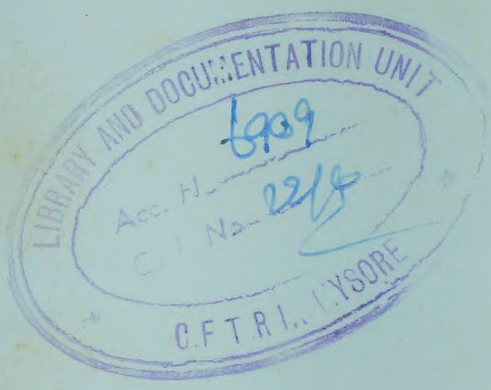

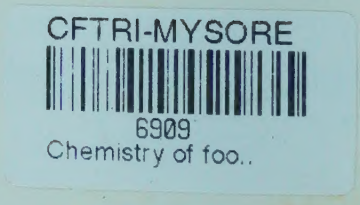

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PREFACE

The purpose of this book is to present the principles of food chemistry and nutrition with as many of the scientific facts as space permits, and as are deemed most important to an effective grasp of the subject. Designed primarily to meet the needs of college classes, it is hoped that the book may also continue to be of service to other readers who are interested in the kinds and amounts of substances needed in our nutrition, the considerations which should underlie our judgments of food values, and the choice and use of foods for the nutritional improvement of life.

Recent research in the chemistry of nutrition has brought to light many new facts, including several discoveries and chemical identifications of previously unknown vitamins, and also has greatly deepened our insights into fundamental principles. Nutrition has been found to play an important part in the maintenance of the body's reputed "steady states" (its relatively constant internal environment). Yet also it is now found that this internal environment is not so rigidly fixed as had been supposed, and is influenced for better or worse by what we take into the body as food, even within the range of conditions universally accepted as normal.

In order thoroughly to incorporate the recent advances into this new edition, every chapter has been revised, several of them rewritten, a new chapter on folic acid, vitamin B_{12}, and the citrovorum factor has been inserted, and the last chapter has been replaced by two new chapters dealing respectively with trends of food consumption and improvement of already normal nutrition.

The present text aims to provide the subject matter for a thorough study of the topics included. Throughout, it has been an outstanding aim so to use clarity and conciseness as to enable the

student to see both more clearly and more deeply into each topic included in this edition. Those readers who want "everything" are referred to the reading lists at the ends of the chapters, which, because of the rapid growth of subject matter in the chemistry of food and nutrition are an ever more important feature of this book.

The discussions of nutritional needs and the tables of nutritive values have been revised in accordance with the current dietary recommendations of the National Research Council and studies of food values by the Federal Government. Fortunately the opportunity for this revision comes at a time when both official and independent publications on food values can be evaluated with more confidence than at any other time within a generation. Hence we may hope that our present judgments may have a better "life expectation" than any of their predecessors could have had.

Throughout the book, special attention has been given to the teaching task which seems of outstanding importance at the present time; namely, to show the far-reaching significance of recent discoveries of fact and advances of fundamental concepts in the science of nutrition, while at the same time safeguarding against exaggerated impressions. Conciseness, readability, and teachability have been kept in mind throughout. In fact, the present edition may be said to reflect forty years' teaching experience and resultant perennial revision of this rendering of the subject.

While the author alone is responsible for any faults of omission or commission, he would gratefully acknowledge his indebtedness to M. E. Bal, M. L. Caldwell, H. L. Campbell, A. P. Doerschuk, C. G. King, C. S. Lanford, Grace MacLeod, C. S. Pearson, M. S. Ragan, J. M. Schwank, W. B. Sherman, C. M. Taylor, and A. W. Thomas for their generous collaboration.

H. C. S.

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CHEMISTRY OF FOOD AND NUTRITION

Chapter 1

GENERAL INTRODUCTION

"Chemistry is the central science." It links the mathematical with the natural sciences and partakes of the properties of both.

Moreover, it is well to remember that all so-called divisions of science into sciences are only man-made: Nature recognizes no such grounds for separation of scientific subject matter. There can be no real boundaries between chemistry, physics, and physiology, for each overlaps both of the others.

The interpenetration of chemistry and physics has been a matter of course in both teaching and research for over a generation, and with epoch-making results too familiar to need recounting. More recently has come the realization that chemistry is chemistry whether studied *in vitro* or *in vivo,* and is greatly enriched by broadening on both its physical and its physiological sides. This broadening of the chemical point of view also adds greatly to its effectiveness in human service.

The objective of scientific research has been well characterized as "to render more intelligible the world in which we live." From this point of view, the two grand divisions of scientific insight are: into the structure of matter; and into the processes of life. Among modern developments adding to our insight into the processes of life, enabling us to understand these processes better, and increasingly to control and improve them, one of the most important is the chemistry of food and nutrition.

Thus the subject appeals both to *the spirit of wonder* and to *the spirit of service.* It functions importantly in the two-fold way indicated by Francis Bacon when he said that science is both for the enlightenment of the mind and for the amelioration of man's estate. Both these aspects of science minister to our comfort and hap-

piness: both help us, as Whitehead put it, "to direct our actions to achieve predetermined ends." Higher health and longer life bring opportunity for greater cultural achievement.

There are also professional reasons for the treatment of our present subject as a branch of chemistry sufficiently developed to have its own literature.

From the viewpoint of the functioning of science in the world of affairs, it is highly significant that in many countries the food industries far outrank other industries economically. It has been estimated, for instance, that in the United States, food products equal or exceed in economic value the total of all other farm products plus all the products of the mines and forests. And chemistry functions largely even in those of the food industries which may not be considered as primarily chemical: for they employ many chemists, and also their products come under chemical inspection and control in the interest of the consumer as they pass into commerce.

From the viewpoint of consumption and cost of living, it is still true for the majority of the world's people, and nearly true for the average American family, that, "Half the struggle of life is a struggle for food." For the more fortunate minority, food does not demand so large a percentage of the income; but at the economic level upon which the majority of families still live, about as much of effort or earnings must be spent upon food as upon all other items in the cost of living, if health and efficiency are to be maintained. This fact calls for a large amount of chemical service in the food work of today. It also calls for the teaching of food values in the schools and colleges and directly to the public. And it has stimulated widespread chemical research and education in the principles of nutrition, the nutritive values of foods, and the use of our knowledge of foods and nutrition in the improvement of health and efficiency.

Major Aspects of This Study

(1) At the turn of the century, the outstanding news in nutrition was that relating to "the man in the copper box," *i.e.*, to the experi-

ments with the respiration calorimeter by means of which the energy relations of nutrition were being established, quantitatively and directly on man, and the foundations thus laid for the building of human nutrition to the pattern of an exact science. These respiration calorimeter experiments were markedly successful. Results of high precision were obtained; and the agreement between the direct measurements of energy (appearing finally in the form of heat) and the computed heat equivalents of the measured chemical exchanges was so close that it became possible (as described in Chapters 8 and 9) to use much simpler and less expensive methods, so-called indirect calorimetry, thus extending the energy aspect of the chemistry of nutrition into the study of everyday food problems (Chapter 10).

(2) Largely simultaneously with this development of the study of energy relations, but reaching its climax a little later, came a truly epoch-making period of experimental enlightenment in the nutritional chemistry of the proteins and in the protein chemistry of nutrition. The proteins which had been studied as chemical individuals and with respect to their amino-acid constitution, were now fed experimentally, first one at a time, then as supplemented by other proteins or by individual amino acids; and thus the chemical natures were correlated with the nutritional functions and relationships (Chapters 4, 5, 7, and 11). Through such work it has been found that some of the amino acids which the food proteins yield are more or less interchangeable, while some are individually indispensable—"nutritionally essential" in the sense that they must each be supplied by our nutriment. It has also been found that food proteins or their amino acids are the antecedents or precursors of several of the body's catalysts which are necessary in order that the chemical reactions involved in digestion and nutrition shall run fast enough to support the life process.

(3) Gradually, during the past half century, in part following upon the protein developments just mentioned and in part independently, there has come an awakening to the importance of mineral elements in nutrition, and correspondingly in the nutritive values of foods. The fact that some of the mineral elements are essential to the anatomical structure and the nutritional functioning of

the body has long been known. But for some time the investigations of this aspect of human nutrition consisted of relatively few and rather primitive experiments with diets in which the mineral elements were so drastically reduced that the results appeared rather artificial and failed to bring realization of the truly scientific and practical importance of these elements in normal nutrition and in food values. Within recent decades, however, systematic work has largely established the quantitative needs for these elements in nutrition, and their quantitative distribution among the chief articles and types of food (Chapter 12–16, 28–34). Some of these mineral elements are often limiting factors in life processes and important in food values because of such uneven distribution among foods that a food supply chosen freely but without scientific guidance may be in real danger of shortage of one or more of these elements, even if acceptable to the senses and customs of the consumer and nutritionally adequate in other respects.

(4) Almost simultaneously with the awakening to the significance of the mineral elements in foods and nutrition, and doubtless obscuring it in the minds of many, there has come a dramatically rapid series of discoveries of the existence in foods and the importance in nutrition of a whole group of substances, called the vitamins. We now know that they are not so related in their chemical natures or in their nutritional functions as to constitute a natural group. Scientifically, the different vitamins should not go together under any group name. That they have done so is chiefly the result of two facts: (*a*) They were all discovered through the growing use of the same general type of innovation in chemical work, namely, the use of laboratory animals as instruments and reagents of chemical research; and (*b*) they were discovered, through their nutritional behavior, in too rapid a succession for their actual isolation and structural identification to keep pace. Thus the vitamins constitute the most definitely dated, but perhaps also the most heterogeneous, of these divisions of our subject-matter. While there are interrelations, especially among the vitamins of the B group, in general the story of each vitamin is unique and should be studied separately upon its own merits (Chapters 17–25).

(5) The four preceding sections indicate a four-fold division of the subject *matter* of our study, in the literal sense of the materials which the food must furnish for the support of the nutritional process. Thus it may be said that any present-day study of nutrition, to be worthy of the name, must stand four-square upon full recognition of energy, protein, mineral elements, and vitamins. Now while each of these is needed as such, and is in that sense independently essential, yet they do not function in isolation: and the interrelationships are so important as to constitute a fifth general aspect of the chemistry of food and nutrition. Thus, as briefly mentioned above, the food proteins furnish amino acids which serve, not only as building materials for the construction of body tissue in the usual sense of the term, but also as precursors from which the body makes the catalysts which expedite the series of reactions involved in bringing the fuel foodstuffs into the service of the energy needs. For instance, glutathione which appears directly to catalyze the oxidation process, thyroxine and adrenine (epinephrine, adrenaline) which function either to expedite the process or to mobilize material for it, insulin which facilitates some preparatory reaction, and the typical hydrolytic enzymes which bring the organic foodstuffs into forms upon which these other catalysts can act—all these now appear to be, in one way or another, derivatives of amino acids such as result from the digestion of food proteins. Or, again, in hemoglobin the body makes for itself a substance which is outstanding both as a protein and as an iron compound, and which functions both in carrying oxygen for the reactions involved in the energy exchange, and in maintaining the acid-base balance. And as a further example of important interrelationships, there are those of the calcium, the phosphorus, and the vitamins concerned in the construction and maintenance of good bones and sound teeth.

These are only a few of the many interrelationships already well recognized to which more will doubtless be added as knowledge of the nutritional process grows. But with something like forty chemical entities (elements or compounds as the individual case may be) which the food must supply, the theory of combinations and permutations indicates a calculable number of interrelationships

far beyond the possibility of study within a human lifetime. Both for practical reasons and in order to make appreciable progress with theoretical concepts, we need to devote our scientific efforts not equally to all interrelationships or to all conceivable questions as to what happens where in the body, but rather to the problems which we have reason to judge are of greatest significance. And this consideration gives the key to what may be considered a sixth major aspect of our study.

(6) The concept or principle of *natural wholes* as bearing upon problems both of food value and of the nutritive welfare of the entire life-cycle or of a family, community, or nation through successive generations. This aspect of our study seeks to give due recognition to the point of view that in the chemistry of nutrition our most significant concern is with the nutritional reactions or responses of the body as a whole. As the very word organism implies, the living body is an organized, coordinated whole, which may behave as something more than a mere summation of its parts. It is such a coordinated whole that is to be nourished. Hence the true significance of a question in the chemistry of nutrition is often best judged by tests in which one observes the nutritional reaction or reactions of the living body as a whole; and sometimes throughout the whole life cycle. Such experiments have now become practicable through the standardization, in recent years, of suitable laboratory animals as instruments and reagents of testing and research in this branch of chemistry.

However interesting and important it may be to follow individual chemical substances into particular bodily organs and to trace complicated interrelations among the details of the intermediary processes in nutrition—and it is thus that much of our knowledge has been, and must be, won—we are also learning that in nutrition the most conclusive evidence is that obtained from the experiment or closely-reasoned series of experiments, conducted with all possible exactness and the most careful laboratory control, upon the whole animal, often throughout its entire life-cycle, and sometimes even through successive generations.

Such comprehensive methods of experimental appeal to nature,

guided by the principle of the whole, may be regarded as completing a six-fold grouping of the major aspects of the present-day study of the chemistry of food and nutrition. These six aspects may also be regarded as six steps in the progress to our present view; or as six pillar concepts upon and around which the chemistry of nutrition is being built.

A Few Definitions and Explanations

The activities upon which the life of the body depends involve a continuous expenditure of energy and also a never-ceasing exchange of material.

Body substances may be, and often are, drawn upon to meet temporary deficiencies of nutrient intake; but ultimately the body is dependent upon food for the fuel substances (oxidizable organic nutrients) which supply energy; for the substances which make good the exchange of body material; and (either by supplying the actual substances or their precursors) for all those regulatory factors which keep the reactions running at the right rates, and keep the internal conditions of the body within the zones of delicate adjustment which the life process requires.

Thus the chief functions of food are: (1) to yield energy; (2) to build, or to renew, body tissue; (3) to regulate body processes and internal conditions, so as to maintain a right internal environment for life.

The changes which take place in the foodstuffs, after they have been absorbed from the digestive tract, are included under the general term *metabolism.* Although the chemical changes and the energy transformations are of course inseparable, it is more or less customary to speak of the metabolism of matter and the metabolism of energy, and to regard the extent of the metabolism of any material substance as measured by the amounts of its end products eliminated, and the extent of the energy metabolism as measured by the amount of heat, or of heat and external muscular work, which the body has disbursed.

The chemical factors of an adequate food supply may now be

summarized as follows: (1) sufficient of the organic nutrients in digestible forms to yield the needed energy; (2) protein, sufficient in amount and appropriate in kind to yield enough of each of the needed amino acids; (3) adequate amounts and proper proportions of the mineral elements needed; (4) sufficient of each of the essential vitamins.

There may or may not be, in the case of any given nutrient, a considerable difference between the merely adequate and the optimal intake. This is a question for experimental investigation with reference, first, to each nutrient separately. And then, as to the possible influence of variations in the intakes of other nutrient factors.

In attempting to give in the following pages a general view of the chemistry of food and nutrition, it has seemed best to discuss: first, the chemical nature and nutritive functions of the carbohydrates, fats, and proteins; second, the nutritive requirements of the body in terms first of energy, then of protein; third, the inorganic or mineral elements in food and nutrition; and fourth, the vitamins. In general this permits us to progress from the topics which are more familiar to those which are newer or less well worked out.

It is important to keep clearly in mind the fact that the newer knowledge of nutrition supplements but does not supplant the knowledge which had been gained before vitamins were discovered or the importance of the mineral elements was appreciated.

Significantly, it is Lavoisier, the chemist who first insisted effectively upon making chemical work quantitative and chemistry an exact (as well as a natural) science, who is now considered the father of the science of nutrition. He showed the similarity between the oxidation of a suitable organic substance in the body and the burning of a fuel in a flame; and he made quantitative experiments upon the rate of oxidation in the body under various conditions. The present-day study of the energy metabolism thus owes its origin to Lavoisier.

Both from the chemical and from the physiological point of view, the term *nutrition* may be used in a very broad sense. Mendel employed the title, *Nutrition: The Chemistry of Life;* and, physio-

logically, the whole state of material well-being of the living organism is sometimes spoken of as nutritional.

In the pages which follow we shall be content to deal with nutrition in the somewhat more specific sense of the life processes as chemically connected in known and fairly direct ways with the actual nutrients supplied by the food. Even this will be found, on the one hand, to involve something of both structural organic and physical chemistry and, on the other hand, to have many far-reaching relationships to the chemical control of the life process and to the resulting internal environment of health and well-being.

In order to bring out clearly the interrelationships and broader significances we may frequently recur to a given topic in different parts of this book.

Present-day science is strongly imbued with the concept that we live in a dynamic world, and that the chief significance of things is to be found not through static descriptions but through the study of their functioning. From our viewpoint, nutrition is the functional aspect of food chemistry.

General Knowledge of Organic Chemistry Presupposed and the Boundaries of General Biochemistry Respected

In order that the text which follows may be kept within the modest size appropriate to its main purpose, and at the same time maintain a broad viewpoint, the plan throughout is to refrain from repeating here any appreciable amount of the present-day subject-matter of courses and textbooks in general organic chemistry. It is presumed that the student will be prepared to refresh or strengthen his acquaintance with general organic chemistry by turning to its modern textbooks in case of need.

Similarly, this book refrains from much discussion of topics which may be considered as belonging at least equally to general biochemistry, such, for example, as the chemistry of the detailed steps in the intermediary metabolism, or of the detailed molecular structure of many of the vitamins.

Chapter 2

CARBOHYDRATES

Introduction

Of the constituents of the ordinary mixed food of man, the carbohydrates are usually the most abundant and the most economical sources of energy. They are also considered to be the first of the three great groups of organic foodstuffs to be formed by synthesis from simple inorganic substances in plants: "in the long run, all the energy of living matter comes from them." The synthesis of carbohydrates in nature is therefore a logical starting point for the study of the organic foodstuffs.

Green leaves utilize the radiant energy of sunshine to bring about the process of *photosynthesis* in which organic compounds such as sugars and starches are synthesized from carbon dioxide and water. Thus is "bound" or "stored" the energy which later becomes available when any direct or indirect product of photosynthesis is utilized as a fuel foodstuff in the body. Space does not permit a discussion of the photosynthetic process here; though an account may be found through Suggested Readings at the end of this chapter (*e.g.*, Mitchell, P. H., 1950, Chapter 2).

We here review only the few carbohydrates most important in food and nutrition and those only in their simplest relations.

Monosaccharides

The monosaccharides are all soluble, crystallizable, diffusible substances, unaffected by digestive enzymes. All of the four with which we are here concerned—glucose, fructose, galactose, and mannose—are utilized for the production of glycogen in the animal body and

the maintenance of the normal glucose content of the blood; but the individual members of this group differ in natural occurrence, and in some details of nutritional relationship.

Glucose (D-glucose, dextrose, grape sugar, corn sugar, starch sugar, diabetic sugar) is widely distributed in nature, occurring in the blood of all animals in small quantity; * and more abundantly in fruits and plant juices, where it is usually associated with fructose and sucrose. It is especially abundant in grapes, of which it often constitutes 20 per cent of the total weight or more than half of the solid matter. Sweet corn, onions, and unripe potatoes are among the common vegetables containing considerable amounts of glucose.

Glucose is also obtained from many other carbohydrates by hydrolysis either by acids or by enzymes † as in natural digestion, and thus becomes the principal form in which the carbohydrate of the food enters into the processes of nutrition. In the healthy body the glucose of the blood is constantly being burned and replaced. Ordinarily any surplus of glucose absorbed from the digestive tract is converted into glycogen which, as described beyond, is readily reconvertible into glucose. Thus, while other carbohydrates occur in food in greater quantity, glucose occupies a very prominent place, partly because it is so widely distributed in both plants and animals, and partly because it is the form in which most of the carbohydrate material of the food comes actually into the service of the body. It is estimated that (under ordinary conditions) over half the energy manifested in the human body is in this sense derived from the oxidation of glucose.

It is not to be inferred that the body obtains the energy of the glucose by oxidizing it directly as such. There is abundant evidence of an *intermediary metabolism.*

According to the point of view or the stage of the intermediary metabolism under consideration, the focal position may be assigned

* It is convenient to remember glucose as constituting about one tenth of one per cent of the blood; but the normal average is slightly less than has been commonly estimated in the past, there being also present small amounts of other substances which react much like glucose to simple analytical testing.

† Enzymes mentioned without discussion in this and following chapters are discussed in Chapter 6.

to glucose-1-phosphate, glucose-6-phosphate, methylglyoxal (pyruvic aldehyde), or adenosine triphosphate, or to the easily reversible phosphorylation of glucose and glycogen.

The chemistry of glucose is further reviewed in considering its relations to fructose and galactose in the following paragraphs.

Fructose (D-fructose, fruit sugar, levulose) occurs with more or less glucose in plant juices, in fruits, and especially in honey, of which it constitutes about one half the solid matter. It results in equal quantity with glucose from the hydrolysis of cane sugar and in smaller proportion from some other less common sugars. Fructose serves, like glucose, for the production of glycogen. Glucose and fructose are convertible, either one into the other, under the influence of very dilute alkalies. It is not surprising, therefore, that fructose should be converted in the liver into glycogen, which on hydrolysis yields glucose.

Glucose and fructose. A convenient chemical explanation of the mutual interconvertibility of glucose and fructose is that the transformation occurs through the intermediate formation of a di-enol (enediol) as now commonly shown in textbooks of organic and physiological chemistry.

Cyclic ("oxygen bridge," lactone, lactal) forms of glucose. At any given time the glucose present in a solution doubtless exists in *at least* three forms; that is, at least one lactone pair in equilibrium with a little of the open chain aldehyde form.*

Of the five structurally possible lactone pairs, two are well established. These are the *1–4 lactone* (*furanose*), and the *1–5 lactone* (*pyranose*) forms.

Glucose as made in the laboratory, and commercial glucose which is a product of acid hydrolysis of starch, is chiefly in the form of the 1–5 lactone pair; while glucose in nature presumably is chiefly in the form of the 1–4 lactone pair. Certainly both these forms exist and both the 1–4 and the 1–5 formulas are "right." Probably this statement of equilibrium stands equally for the furanose and pyranose forms; and if each is in equilibrium with the aldehyde form they are

* Cf. Conant: *The Chemistry of Organic Compounds*, 3rd Edition, pp. 289–291 (1947).

thus at least indirectly in equilibrium with each other. Hence when we say that the glucose produced *in vitro* is principally the 1–5 form (pyranose) and that of nature is principally the 1–4 form (furanose), it might be more strictly correct to say that it is chiefly the 1–5 form which enters into or emerges from the familiar *in vitro* reactions, while it is chiefly as the 1–4 form that glucose undergoes its well known nutritional reactions such as the transformation of glucose into galactose radicles in the synthesis of lactose in the mammary gland and the breaking of glucose phosphate into three-carbon intermediates when glucose is metabolized in the tissues generally.

The structural chemistry and interrelationships of the sugars, if not already familiar to the reader, may readily be consulted in textbooks of organic and physiological chemistry. See list of Suggested Readings at the end of the chapter.

Bodansky (1938, p. 43) cites P. A. Levene as authority for the view that glucose solutions may contain all of the theoretically possible cyclic structures (lactone forms) in equilibrium with each other. The 1–5 form is believed to exist in larger proportion, but under the influence of agents which act specifically upon *any* one form, this form may enter into reaction; and as it reacts and so is removed from the equilibrium the other forms are changed into it. Thus the products may show which form entered predominantly into reaction under the given circumstances, rather than which form was present in largest amount in the original glucose solution. This view should avoid the confusion sometimes caused by the fact that certain reactions *in vitro* indicate chiefly the 1–5, and certain nutritional reactions chiefly the 1–4 form.

Galactose results (along with glucose) from the hydrolysis of milk sugar, either by acid or by digestive enzyme, and appears, like glucose and fructose, to be convertible into glycogen in the animal body. If glucose and galactose be represented by their (full structural) 1–4-lactone formulas, it will be seen that a very direct stereochemical relationship exists between them so that the fact just noted that galactose goes to form the same glycogen in the body as is formed from glucose, and the further fact that galactose is evi-

dently formed from glucose in the mammary gland, both become readily intelligible. For the difference between glucose and galactose as thus represented lies only in the spatial relation of the oxygen bridge to the rest of the molecule, and this is evidently changed in the mammary gland with resulting transformation of glucose into galactose which then combines with some of the remaining glucose to form lactose. The transformation probably occurs through the enzymic action of something in the gland cell which first forms a combination with glucose and then splits off galactose, the stereoisomeric shift of the oxygen bridge occurring in the course of the formation and subsequent cleavage of the complex intermediate compound which the sugar radicle forms with the enzyme molecule —this latter being itself doubtless a very complex and optically active substance.

Polymeric anhydrides of galactose, known as galactans, occur quite widely distributed in plants; and galactosides, which are compounds containing galactose in chemical combination with radicles of other than carbohydrate nature, are constituents of the brain and nerve tissues. Galactose has also been recognized as a minor constituent of several proteins.

Mannose also, although not prominent in the carbohydrate foodstuffs, has recently been reported as occurring (in traces) in several proteins.

Disaccharides

The three disaccharides here considered are *dihexoses* or *hexobioses* of the formula $C_{12}H_{22}O_{11}$, and are crystallizable and readily diffusible. Sucrose crystallizes anhydrous; maltose and lactose each with one molecule of water, which can be removed by drying at temperatures of 100° and 130° respectively. They are soluble in water; less soluble in alcohol. Lactose is much less soluble than sucrose and maltose. These disaccharides are important constituents of food and are changed to monosaccharides during the process of digestion.

Sucrose (saccharose, cane sugar) is widely distributed in the

vegetable kingdom, being found in considerable quantity, generally mixed with glucose and fructose, in the fruits and juices of many plants. The commercial sources of sucrose are the sugar beet, the sugar and sorghum canes, the sugar palm, and the sugar maple; but many of the common fruits and vegetables contain notable amounts. For example, sucrose is said to constitute at least half the solid matter of pineapples and of some roots such as carrots.

The leading source of sucrose is the sugar cane, the second is the sugar beet, and all others are of only local or insignificant commercial importance compared with these. Needless to say, there is a large literature on sugar production, sugar refining, and the sugar trade. Besides the special books and journals devoted to sugar, several of the handbooks on industrial chemistry have sections on the sugar industry, and a chapter on the subject is included in the writer's *Food Products.*

The per capita consumption of sugar per year in the United States increased from about 10 pounds in the 1820s to about 100 pounds in the 1920s and most of the time since. The *difference* between the level of sugar consumption of our generation and that of four generations ago probably covers at least one tenth of our total food calorie intake, and correspondingly displaces the equivalent of one tenth of all nutrients other than carbohydrate which we would otherwise get in eating to meet our energy requirement. Is it a matter of indifference to have our intakes of the other factors of our nutrition thus reduced? Or would we profit by having a food supply richer in some at least of the mineral elements and protein and vitamin values? Would we be better off if a part of the producers' land and labor, and of the consumers' money, now devoted to sugar, were devoted instead to increasing the production and consumption of foods which together with their calories furnish us also other nutrients needed to balance our dietary? Facts bearing upon such questions will be developed in many of the chapters which follow.

On hydrolysis, a molecule of sucrose yields one molecule each of glucose and fructose. These sugars all rotate the plane of polarized light: sucrose and glucose to the right (+), and fructose to the

left (−). The terms *dextrose* and *levulose*, synonyms for glucose and fructose respectively, arose from this behavior of the sugars in rotating the plane of polarized light to the right and left. Since at ordinary temperatures the fructose rotates more strongly to the left than the glucose does to the right, the result of the hydrolysis of sucrose is to change ("invert") the sign of rotation from + to −. For this reason the hydrolysis of cane sugar is often called *inversion*, and the resulting mixture of equal parts of glucose and fructose is known as *invert sugar*.

Sucrose is very easily hydrolyzed either by acid or by the *sucrase* (*invertase* or "inverting" enzyme) of yeast or of intestinal juice. So far as known, neither the saliva nor the gastric juice contains any enzyme capable of hydrolyzing cane sugar, and any hydrolysis of sucrose which takes place in the stomach is believed to be due to the presence of hydrochloric acid or to the regurgitation of intestinal juices into the stomach, which may occur more commonly than is generally realized. In the intestine, sucrose is split by the sucrase ("invertase") of the intestinal juice, and the resulting glucose and fructose are absorbed into the portal blood.

Lactose (milk sugar) occurs in the milk of all mammals, constituting usually from 6 to 7 per cent of the fresh secretion in human milk and 4.5 to 5 per cent in the milk of cows and goats. At the time of parturition or if the milk is not withdrawn from the udder, some lactose may occur in the urine. If in such a case the mammary glands are removed, the percentage of glucose in the blood increases, and glucose (but no lactose) may appear in the urine. These observations indicate that lactose is formed in the mammary gland and probably from the glucose brought by the blood. The chemistry of the process has been discussed briefly in the section on galactose above. For fuller discussion and further accounts of experimental study, see Watkins (1928).*

Lactose is less sweet and much less soluble than sucrose, dissolving only to the extent of about 1 part in 6 parts of water. When hydrolyzed either by heating with acid or by an enzyme, such

* References for suggested reading, whether specifically cited in the text or not, are given at the ends of the respective chapters.

as the *lactase* of the intestinal juice, each molecule of lactose yields one molecule of glucose and one of galactose. Hence the lactose eaten is absorbed, not as such, but as a mixture of equal parts of glucose and galactose. Herter found lactose to be less subject tc fermentation in the stomach than is sucrose. Also, because of the much lower solubility, there is less danger of direct irritation of the stomach membrane by lactose than by sucrose. Mathews has suggested that the occurrence in milk of lactose, a sugar having the galactose radicle, may be of special significance as a source of material for the synthesis of the galactosides of the brain and nerve tissues of the rapidly growing young mammal. Furthermore, much research, summarized and reaffirmed by Associates of Rogers (1935), has shown that the taking of liberal quantities of lactose in the food is for some people an important aid to the maintenance of good intestinal conditions, because the lactose is especially favorable to the development of desirable types of intestinal bacteria, notably the *Bacillus* (*Lactobacillus*) *acidophilus.*

In order that this latter advantage may be had along with greater solubility and apparent sweetness for those who desire it, *beta-lactose* * has been studied scientifically and made commercially available.

Maltose (milk sugar) is formed from starch by the action of diastatic enzymes (amylases) and is therefore an important constituent of germinating cereals, malt, and malt products. It is also formed as an intermediate product when starch is digested in the human or other animal body, or hydrolyzed by boiling with dilute mineral acid as in the manufacture of commercial glucose.

In animal digestion, maltose is formed by the action of the ptyalin of the saliva (salivary amylase) or of the amylopsin of the pancreatic juice (pancreatic amylase) upon starch or dextrin. The maltose-splitting enzyme of the intestinal juice readily hydrolyzes maltose to glucose. Maltose is also readily and completely hydrolyzed by

* Lactose, glucose-β-galactoside, exists in α- and β-forms corresponding to α- and β-glucose. The ordinary "milk sugar" of commerce is α-lactose. However by allowing solutions of lactose to crystallize above 90°, the β-isomer is obtained.

boiling with dilute mineral acids. In either case each molecule of maltose yields two molecules of glucose.

Polysaccharides

The polysaccharides are all insoluble in alcohol. Some dissolve in water in the sense that they form colloidal dispersions which will pass through filter paper; some swell and become gelatinous; some are apparently unchanged. The members of greatest importance in nutrition are starch and glycogen, the typical reserve carbohydrates of plants and animals respectively, and the dextrins which are intermediate products in the hydrolysis of starch.

Starch, approximately $(C_6H_{10}O_5)_x$, is the form in which most plants store the largest part of their carbohydrate material, and is of great importance as a constituent of many natural foods and as the source of dextrins, maltose, and glucose. Starch is found stored in the seeds, roots, tubers, bulbs, and to some extent in the stems and leaves of plants. It constitutes one half to three fourths of the solid matter of the ordinary cereal grains and at least three fourths of the solids of mature potatoes.

Unripe apples and bananas contain much starch which is to a large extent changed into sugars as these fruits ripen; while, on the other hand, young tender corn (maize) kernels and peas contain sugar which is transformed into starch as these seeds mature.

Starch granules are scarcely affected by cold water; on warming they absorb water and swell. Finally the starch passes into a condition of colloidal dispersion or semi-solution, "starch paste." Starch which has been heated in water (either admixed or naturally present with the starch as in a potato) until the granules are ruptured and the material more or less dispersed is very much more rapidly hydrolyzed by digestive enzymes than is raw starch. The starch-digesting enzymes (*amylases*) of the saliva and pancreatic juice change starch through dextrin to maltose; and (as noted above) a digestive ferment of the intestinal juice changes the maltose to glucose. Acid hydrolysis also changes starch through dextrin and maltose to glucose.

Thus far we have followed the usual custom of speaking of starch as if it were a single substance of purely carbohydrate nature. Actually, however, the strictly carbohydrate material of the starch is combined with a very minute proportion of one or more acid radicles, sometimes fatty acid and sometimes containing phosphorus. Also starch yields, even under such treatment as would not be expected to disrupt a chemical compound, two substances: α-amylose (also called amylopectin) which contains the small amount of non-carbohydrate material, and which forms on heating in water a viscous opalescent paste; and β-amylose (also called amylose) which forms when heated in water a clear limpid solution which gives a pure blue color with iodine. The starch-digesting enzymes hydrolyze both α-amylose and β-amylose, but not necessarily at the same rate, and in some cases at very different rates.

"Soluble starch," largely used for laboratory experiments, is commonly prepared by soaking raw potato starch in cold hydrochloric acid (about 7 per cent HCl) for several days, and then washing with cold water, or by treating starch with alkali under very carefully regulated conditions.

Dextrins, approximately $(C_6H_{10}O_5)_x$, or $(C_6H_{10}O_5)_x \cdot H_2O$, are formed from starch by the action of enzymes, acids, or heat. The term *dextrin,* even if used in the singular, must be understood as designating a group rather than an individual substance. Small amounts of dextrin are found in "resting," and larger amounts in germinating, cereals. Malt diastase, acting for some time upon starch in fairly concentrated solution, yields usually about one part of dextrin to four of maltose. Dextrins thus formed by hydrolysis are sometimes called *hydrolytic dextrins.* Commercial dextrin, the principal constituent of "British gum," is obtained by heating starch, either alone or with a small amount of a dilute, or of a weak,* acid. The dextrins formed essentially by dry heating are sometimes called *torrefaction dextrins.*

The dextrins are much more soluble than the starches; and dextrin molecules, while doubtless very large and complex, are probably

* Students should keep this chemical distinction in mind, even if chemists do sometimes fail to observe it.

much smaller in size than the starch molecules from which they are derived.

The digestion of dextrin has already been mentioned in connection with that of starch, both saliva and pancreatic juice forming dextrin during the digestion of starch and acting upon it with the production of maltose. Complete hydrolysis of dextrin, as by boiling with acid, yields glucose as the final product.

The work of Rettger indicates that dextrin is, like lactose, a favorable medium for the nourishment of the *Lactobacillus acidophilus* in the digestive tract, so that liberal proportions of dextrin and lactose in the food are conducive to a good intestinal hygiene.

Glycogen $(C_6H_{10}O_5)_x$ plays much the same role in animals which starch plays in plants, and is sometimes called *animal starch.* It is a white, amorphous powder, odorless and tasteless, which swells and apparently dissolves in cold water to an opalescent colloidal dispersion which is not cleared by repeated filtration, but loses its opalescence on addition of a very small amount of potassium hydroxide or acetic acid. Water solutions (dispersions) of glycogen are readily precipitated by alcohol. When treated with iodine they react yellow-brown, red-brown, or deep red. Complete hydrolysis of glycogen yields glucose only, as an end-product.

Glycogen occurs in the lower as well as the higher animals, and in nearly all parts of the body, but reaches its highest concentration in the liver. The amount of glycogen in the liver depends to a great extent upon the condition of nutrition of the body. But we no longer think of body glycogen merely as material in "dead storage" or playing only a passive part, especially since Cori (1941, p. 139) emphasized the fact that glycogen undergoes a reversible phosphorylation which "requires little or no energy and can therefore occur directly with inorganic phosphate." Later papers by Cori and his coworkers have amplified and clarified the chemistry of the carbohydrate metabolism.

Cellulose and hemicelluloses are polysaccharides still less soluble or less readily hydrolyzed than those above described. They contribute little if anything to the strictly nutritive values of foods, but their presence in proper proportion in the diet as a whole is favor-

able to the mechanics of digestion and the hygiene of the digestive tract. All such material not dissolved by the digestive enzymes is sometimes called "roughage"; but more accurately speaking it is only some forms of fiber which are rough, while other forms of cellulose and nearly all forms of hemicellulose are relatively soft and smooth, yet contribute a desirable *bulk* to the food mass and its undigested residue. Every normal dietary should contain enough indigestible fiber (chiefly celluloses and hemicelluloses) to give the digestive canal "its daily scrubbing"; but to what extent this should be real roughage, and to what extent the soft smooth forms of cellulose and hemicelilulose should be preferred, is a matter which may differ largely with individuals. An amount of real roughage (or a degree of real roughness) which is advantageous to one person may be excessive for another. In a population of many millions there very likely may be a considerable number of people who have treated themselves to too much roughage; and there may at the same time be an equal or larger number of people whose ordinary food habits give them less bulk of undigested residue than is best. In cases of doubt, one may carefully experiment (1) with increased intakes of soft cellulose and hemicelluloses as in ordinary fruits and vegetables, and (2) with a rougher form of fiber as in bran. To some extent, the effects of different chemical components of vegetable fiber are being studied experimentally. There is also growing recognition of the value of liberal amounts of fruits and their juices in promoting intestinal regularity.

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Chapter 3

FATS AND LIPOIDS: LIPIDS (LIPINS)

Lipids is the name preferred by Bloor (1943) as the general term to cover the fats and related substances. He defines lipids as a group of naturally occurring substances consisting of the higher fatty acids, their naturally occurring compounds, and substances found in natural association with them. He classifies them essentially as follows:

Bloor's Classification of Lipids

Simple lipids. Esters of the fatty acids with various alcohols.

Fats—esters of the fatty acids with glycerol.

Waxes—esters of the fatty acids with alcohols* other than glycerol.

Compound lipids. Esters of the fatty acids containing groups in addition to an alcohol and fatty acid.

Phospholipids (phosphatides)—substituted fats containing phosphoric acid and nitrogen, *e.g.*, lecithin, cephalin, sphingomyelin.

Phosphatidates—phospholipids minus the organic bases.

Glycolipids—compounds of the fatty acids with a carbohydrate and containing nitrogen but no phosphoric acid, *e.g.*, cerebrosides.

Sulfolipids—lipid molecules containing sulfuric acid radicles.

(Aminolipids, etc., not yet well characterized.)

Derivatives of lipids.

Fatty acids of various series.

Alcohols, including glycerol—mostly large molecular alcohols, found in nature combined with the fatty acids and which are soluble in the fat solvents—cholesterol ($C_{27}H_{45}OH$), myricyl alcohol ($C_{30}H_{61}OH$), cetyl alcohol ($C_{16}H_{33}OH$), etc. (The term *sterols* has often been applied to complex hydroaromatic secondary alcohols such as cholesterol and ergosterol. See Bills, 1935.)

* In the familiar waxes, these are alcohols of high molecular weight, and in most cases are "monatomic," *i.e.*, with only one hydroxyl group in the molecule.

Some hydrocarbons, *e.g.*, squalene. (And some might include here the carotenes—Chapter 23.)
Some bases, *e.g.*, choline—Chapter 22.

The characterization of the above substances as naturally occurring means simply that they have been found in nature. Of course, many of them have also been synthesized.

The most readily noticeable characteristic of the lipids is their solubility in the "fat solvents" such as ether, chloroform, benzene, gasoline, carbon tetrachloride, as contrasted with their relative insolubility in water. This sets them off in an obvious way from the carbohydrates, proteins, and mineral matters. Yet some of the lipids such as the lecithins form filtrable dispersions with water, and also differ from ordinary fats in the extent to which they dissolve in alcohol, in acetone, or even in ether.

Fats (Glycerides)

Fats are glyceryl esters of fatty acids, and since glycerol is a triatomic alcohol and the fatty acids are monobasic, a normal glyceride is a triglyceride and on hydrolysis yields three molecules of fatty acid and one molecule of glycerol. Thus, for example:

$$\underset{\substack{\text{Stearin}\\\text{(glyceryl tristearate)}}}{C_3H_5(C_{18}H_{35}O_2)_3} + 3\ H_2O \longrightarrow \underset{\text{Glycerol}}{C_3H_5(OH)_3} + 3\ \underset{\text{Stearic acid}}{C_{18}H_{36}O_2}.$$

When the splitting of the fat is brought about by means of an alkali, the corresponding products are glycerol and the alkali salt of the fatty acid; and since alkali salts of the fatty acids are commonly known as soaps, this reaction is usually called the saponification of the fat.

The fats are, as implied in the above definition, a structurally distinct group of chemical compounds, and the term applies equally to the solid and the liquid members of this group. As a matter of convenience, however, the liquid fats are often called *fatty oils.* The fatty oils are also sometimes called *fixed oils,* because a spot made by dropping a fatty oil on paper cannot be removed by dry-

ing (as can a volatile oil), nor by washing with water (as can glycerol).

The fat of a food (with more or less of the phosphatides and sterols) may be separated from the other chief components by drying the food and extracting the dry material with pure ether. After the fat has been completely dissolved away from the other constituents of the food, it can be recovered from the solvent by evaporating the latter at a relatively low temperature. This is the method commonly used to estimate the percentages of fat in foods and to obtain small portions of fat for examination. It must be noted, however, that the fat thus obtained is not always pure in the sense of consisting entirely of substances meeting the definition of fat as given above; but will contain, along with the fat, any other ether-soluble substances which were present in the food, and may contain substances which, while not appreciably soluble in ether alone, are dissolved by mixtures of ether and fat. It is, therefore, somewhat more accurate to speak of the material extracted by ether as *ether extract* rather than as *fat*, and it will often be found so designated in statements of analytical results.

The food fats of commerce have been separated from the materials in which they naturally occurred, not usually by solvents as above described, but more often by mechanical means such as the churning of butter and the pressing-out of olive, cottonseed, peanut, or soybean oil. In either case the naturally occurring fat-soluble substances remain dissolved in the separated fat, unless removed or destroyed by some subsequent refining process.

The separated forms of fat occurring as such in commerce are often called the "visible" fat supply in contradistinction from the fat contained in other foods such as meats, eggs, milk, or cheese.

Shortage of fats during each of the World Wars has increased the utilization of oil-bearing seeds as sources of commercial food fats. In 1942–44 soybeans came into greatly increased prominence as a source of commercial food fat in the United States. Peanut oil has also been growing in prominence.

Burr estimates that the British and American people in normal times consume over 100 grams of fat per capita per day, while many

Oriental people consume only about 30 grams or less. To study the question of the actual need of fat in human nutrition, Brown, Hansen, Burr, and McQuarrie (1938) maintained an adult human subject for a period of 6 months on a nearly fat-free diet "without demonstrable harm." As the subject was one of themselves, we may assume that any significant sign of harm would have been noticed and recorded; also that no other fat was consumed than that contained in the experimental diet, which was less than 2 grams (or 0.03 gram per kilogram of body weight) per day.

However, in comparing the findings of this experiment with those of work on rats, it is to be remembered that, considered as a segment of the life cycle, 6 months in the life of a man are equivalent only to about 6 days in the life of a rat. Rats placed on this diet when 4 weeks old ("end of infancy" in the rat) showed abnormality within 8 weeks, which corresponds to about 4 to 5 years of human life.

These investigators considered the previous work of Burr and Burr (1929), McAmis, Anderson, and Mendel (1929) and Evans and Lepkovsky (1932) as having established (*a*) that some animal species are unable to synthesize linoleic and other highly unsaturated fatty acids, and (*b*) that such fatty acids are essential for the proper nutrition of rats. Previous studies of nearly fat-free diets with human subjects had been confined to a few infants, some of whom developed eczema, while others showed decrease in the degree of unsaturation of the fatty acids of the blood serum, similar to that which had been found in rats suffering from fat deficiency and in infants with eczema (Hansen, 1937, and others).

The man who served as subject for their above-mentioned 1938 research remained "clinically well" throughout the 6 months of the experiment, and at no time did any of the foods of his extremely low-fat diet become distasteful. Although the energy value of the experimental diet (2500 Calories a day) was supposedly adequate, and was uniform throughout the experiment, there was during the first 3 months a decline of 14 pounds in body weight, after which it remained constant.

The outstanding effect of the extremely low-fat diet upon the

man was to cause a decrease in the degree of unsaturation of his serum lipids, as observed regularly in rats subjected to fat-deficiency, and occasionally in children with eczema.

Brown, Hansen, Burr, and McQuarrie therefore believe that qualitatively the essential effect of fat deprivation is the same for the human species as for the rat and that a man could not be expected to remain in good health on an extremely low-fat diet for the whole or a major part of a normal lifetime.

Yet with our national tendency to eat three times as much fat per capita as do many other peoples, the same question arises with respect to fat as to sugar, whether it is wisest to take so much of our food-energy in forms so poor (or lacking) in protein, mineral elements, and some of the vitamins.

And, as we shall also see in Chapter 6, the level of fat in the dietary raises a question with reference to the time relations of the digestive process.

Fatty Acids

The greater number of the naturally occurring fatty acids belong to a few homologous series. The series to which stearic acid belongs may be represented by the general formula, $C_nH_{2n}O_2$. Nearly all the naturally occurring fatty acids have even numbers of carbon atoms in the molecule. The members of greatest importance are as follows:

Butyric acid, $CH_3(CH_2)_2COOH$, occurs as glyceride to the extent of about 5 to 6 per cent in butter and in very small quantities in a few other fats.

Caproic acid, $CH_3(CH_2)_4COOH$, is obtained from goat and cow butter and coconut fat.

Caprylic acid, $CH_3(CH_2)_6COOH$, is obtained from coconut oil, goat and cow butter, and human fat.

Capric acid, $CH_3(CH_2)_8COOH$, is obtained from coconut oil, goat and cow butter, and the fat of the spice bush.

Lauric acid, $CH_3(CH_2)_{10}COOH$, occurs abundantly as glyceride in the fat of the seeds of the spice bush, and in smaller proportions in butter, coconut fat, palm oil, and some other vegetable oils.

Myristic acid, $CH_3(CH_2)_{12}COOH$, is obtained from nutmeg butter, coconut oil, butter, lard, and many other fats, as well as from spermaceti and wool wax.

Palmitic acid, $CH_3(CH_2)_{14}COOH$, occurs abundantly in a great variety of fats, both animal and vegetable, including many fatty oils, and also in several waxes, including spermaceti and beeswax.

Stearic acid, $CH_3(CH_2)_{16}COOH$, is found in most fats, occurring more abundantly in the solid fats and especially in those having high melting points.

Butyric acid is a mobile liquid, mixing in all proportions with water, alcohol, and ether; boiling without decomposition; and readily volatile with steam. With increasing molecular weight, the acids of this series regularly show increasing boiling or melting points, and decreasing solubility and volatility. For ordinary temperatures the dividing line between liquids and solids falls at about caproic acid. Stearic acid is a crystalline solid, insoluble in water, and only moderately soluble in alcohol and ether.

Unsaturated Fatty Acids

These are unsaturated compounds in that each molecule contains one or more ethylene linkage or "double bond," and can take up halogen by addition to form a saturated compound; or, with a suitable catalyst, hydrogen may be added (hydrogenation). The relative number of double bonds is measured analytically by determining the percentage of iodine which the fat or fatty acid will absorb. This is called the *iodine number.* Thus pure oleic acid (mol. wt. 282) absorbs 2 atoms of iodine, giving an iodine number of 90; pure linoleic acid (the corresponding acid of the $C_nH_{2n-4}O_2$ series) would absorb 4 atoms of iodine to the molecule, giving an iodine number about twice as great; and so on. These unsaturated acids have, as a rule, much lower melting points than the saturated acids containing the same number of carbon atoms; and their fats are correspondingly softer. Such soft fats or fatty oils can be hardened to any desired consistency (up to that of stearin) by hydrogenation, which changes the unsaturated fatty acid radicles into the corre-

sponding members of the more saturated series. This process has been enthusiastically developed commercially and large quantities of oil are now hydrogenated to the consistency of lard substitutes. It remains to be determined how far this is at the expense of the special nutritional value which food fats may owe to the presence of some of the more highly unsaturated fatty acid radicles, and whether hydrogenated fats should bear some warning on this nutritional ground.

Oleic acid, $C_{18}H_{34}O_2$, occurs as glyceride in nearly all fats and fatty oils and is by far the most abundant member of the series. Many of the typical oils of both animal and vegetable origin, such as lard oil and olive oil, consist largely of glycerides containing oleic acid radicles.

Erucic acid, $C_{22}H_{42}O_2$, has long been known as obtainable from the seed oils of cruciferous plants, such as the commercial fatty oils of rapeseed and of mustardseed. More recently it has been found also in marine animal oils (Hilditch).

Oleic and erucic acids are the best-known members of the series $C_nH_{2n-2}O_2$.

Acids of the series $C_nH_{2n-4}O_2$, $C_nH_{2n-6}O_2$, and $C_nH_{2n-8}O_2$ have now been found to occur as glycerides in many fats. *Linoleic acid*, $C_{18}H_{32}O_2$, and *linolenic acid*, $C_{18}H_{30}O_2$, take their names from having been first found in linseed oil; *arachidonic acid*, $C_{20}H_{32}O_2$, occurs in the lipids of the brain, liver, and other organs, and of egg yolks. These are but examples; probably many other fatty acids of these series may occur in natural fats.

It is now generally regarded as established that some unsaturated fatty acid or acids is or are *nutritionally essential* in the sense in which this term has long been applied to some of the amino acids in connection with protein metabolism, *i.e.*, that these acids either are not synthesized in the body or not rapidly enough to meet its needs for best results, so that, for optimal nutritional well-being, they must be furnished in some form in the nutriment. Of course these nutritionally essential substances need not exist free in the food: the nutritionally essential amino acids exist in the food chiefly as constituents of food proteins; and the nutritionally essential fatty

acid or acids exist in fats chiefly as constituents of some of the food fats.

Recognition of the special nutritional importance of some of the more highly unsaturated fatty acids has been followed by reinvestigation of several food fats as to the presence of such acids, with the result that they are now being reported where previously they had escaped attention. Butter fat has been found by Hilditch and Sleightholme (1930) to contain linoleic acid, while Bosworth and Brown (1933) add decenoic ($C_{10}H_{18}O_2$) and tetradecenoic ($C_{14}H_{26}O_2$), Eckstein (1933) adds linolenic, and Bosworth and Sisson (1934) find that arachidonic acid is also present. See also Green and Hilditch (1935).

Simple and Mixed Triglycerides

Triglycerides in which the three fatty acid radicles are of the same kind are known as *simple triglycerides*. Tristearin, triolein, tripalmitin, are examples of simple triglycerides. A *mixed triglyceride* is one in which the three fatty acid radicles are not all of the same kind. For example, distearo-olein (having two radicles of stearic and one of oleic acid), dioleo-palmitin (having two radicles of oleic and one of palmitic), and stearo-oleo-palmitin (having one radicle each of stearic, oleic, and palmitic acids) all are mixed triglycerides.

Taking account of the structurally possible isomers, it can be calculated, according to the principle of combinations and permutations, that the number of mixed triglycerides may increase rather rapidly with the number of kinds of fatty acid concerned; for example, with ten kinds of fatty acid, ten simple triglycerides and 540 mixed triglycerides are possible.

The simple triglycerides corresponding to the common fatty acids are all known; but naturally not all of the many possible mixed triglycerides have been prepared. There is, however, no reason to doubt that the mixed triglycerides compose a large part of most natural fats.

When we consider the important extent to which lipids enter into

the composition of body tissues, fluids, and membranes, and modify their properties, we see that the probable existence of large numbers of triglycerides and of their natural derivatives may be of very great significance in connection with the differences in physico-chemical structure and properties which are so influential in determining the transport and utilization of nutritive material in and through the fluids and membranes of the body.

Formation and Composition of Natural Fats

Fats are formed both in plants and in animals. The conditions which determine fat formation, and the character of the fat formed in different species and under different conditions, are better known than the chemical steps involved in the process. It is hardly necessary to mention the fact that the true fats are composed of the same three chemical elements of which the carbohydrates are composed (carbon, hydrogen, and oxygen) and that since the fats contain less oxygen and more carbon and hydrogen than the carbohydrates, they constitute a more concentrated form of fuel or a compact and lighter medium for the storage of energy for future use. Also, in the animal body, stored carbohydrate is accompanied by much more water than is stored fat.

Hilditch and others have emphasized the general trend toward higher proportions of unsaturated, and then of more highly unsaturated, fatty acids in the fats formed in the cooler regions, by plants as well as by animals.

Formation of Fat from Carbohydrate. From the standpoint of our present knowledge it would seem that the readiness with which farm animals are fattened on predominantly carbohydrate food should have been sufficient to convince early observers; but this evidence was for some time overlooked because of the idea, then prevalent, that simpler substances are built up into more complex compounds only in the plant, and not in the animal organism. Later, chemists learned that animals as well as plants may show great synthetic activity and there is now abundant evidence that the animal body synthesizes fat from carbohydrate.

The most obvious method of demonstrating the conversion of

carbohydrate into fat is that followed by Lawes and Gilbert. Several pigs of the same litter and of similar size were selected; some were killed and analyzed as controls, while the others were fed on known rations and later weighed, killed, and analyzed to determine the kinds and amounts of material stored in the body. In several cases the amounts of fat stored during such feeding trials were found to have been much larger than could be accounted for by all of the fat and protein fed, so that at least a part, and in some cases the greater part, of the body fat must have been formed from the carbohydrate of the food. Many similar experiments have been made, and the transformation of carbohydrate into fat has been demonstrated by this method in carnivorous as well as herbivorous animals.

It has also been shown that carbohydrates contribute to the production of milk fat. Jordan and Jenter kept a milch cow for fifty-nine days upon food from which nearly all of the fat had been extracted. During this period about twice as much milk fat was produced as could be accounted for by the total fat and protein of the food, and in addition the cow gained in weight and her appearance showed that she had more body fat at the end than at the beginning of the experiment.

Instead of determining directly the fat formed in the animal fed on carbohydrate, the production of fat from carbohydrate may be demonstrated by keeping the animal experimented upon in a respiration chamber so arranged that the total carbon given off from the body may be determined and compared with the total carbon of the food. If in such a case the body is found (by the methods explained in later chapters) to store more carbon than it could store as carbohydrate or protein, it is safe to infer that at least the excess of stored carbon is held in the form of fat. Many such experiments upon dogs, geese, and swine have shown storage of carbon very much greater than could be accounted for on any other assumption than that a part of the carbon of the carbohydrates eaten remained in the body in the form of fat.

Further evidence of the transformation of carbohydrate into fat in the animal body is obtained from the respiratory quotient, as explained in Chapter 7.

Composition and Properties of Animal Fat

Usually the nature of the fat found in the body is more or less characteristic of each species or group of closely related species. Herbivora contain as a rule harder fats than carnivora, land animals have harder fats than marine animals, and all warmblooded animals have fats of higher melting points than those found in fishes. The fats of the adipose tissues of different mammals show little variation from an average of: carbon, 76.5 per cent; hydrogen, 12 per cent; oxygen, 11.5 per cent.

Butter fat differs from body fat in containing fatty acids of lower molecular weight (particularly butyric acid, which is fairly characteristic of butter), and so shows a higher percentage of oxygen and lower percentages of carbon and hydrogen. The most abundant acids of butter fat are, however, palmitic, oleic, and myristic, and the ultimate composition is not very greatly different from that of body fats. A sample of milk fat analyzed by Browne showed 75.17 per cent carbon, 11.72 per cent hydrogen, and 13.11 per cent oxygen. For further studies of milk fat and the influence of food upon it, see the work of Hilditch and Sleightholme (1931), of Eckstein (1933), of Bosworth and Brown (1933), of Bosworth (1934), and of Riemenschneider and Ellis (1936).

In discussing the formation of body fat from carbohydrate it was shown that often the greater part of the fat stored is produced in the body from carbohydrate. So striking were the results of some of the experiments demonstrating the synthesis of fat from carbohydrate, that it was even questioned for a time whether any of the fat deposited in the tissues comes from the fat in the food.

Abundant evidence that food fats may contribute to the store of fat in the body has been obtained by feeding characteristic fats or "tagged" or "tracer" fatty acids, and showing that these fats or fatty acids can afterwards be recognized in the tissues of the experimental animals. Experiments of this kind were early made with dogs, using linseed oil, rapeseed oil, or mutton tallow, any of which is distinguishable by the chemical and physical properties of its fatty acids from the fat normally found in the body of the dog.

More recently it has become possible to use fatty acids containing deuterium (heavy hydrogen) in experiments of this kind. Schoenheimer and Rittenberg (1935, 1936), starting with linoleic acid, introduced four atoms of deuterium, then fed this and later determined deuterium in the fats of different parts of the body. Their experiments impressed them with the extent to which the fat fed is carried to the body's fat depots, even when there is no apparent surplus of fat.

The occurrence in the body fat of properties usually characteristic of some particular fat which has been fed has long been known and recognized in establishing standards of purity for fats of animal origin. Thus, the lard obtained from swine which have been fed cottonseed meal shows the characteristic color reactions of cottonseed oil, and more elaborate tests must be made in order to determine whether cottonseed fat has actually been mixed with the lard.

Evidence of the formation of body fat from food fat has been obtained also by experiments upon the total amount of fat formed in the body when the amount and composition of the food eaten were accurately known.

Thus there is abundant experimental evidence that both the carbohydrate and the fat of the food may serve as sources of body fat; and it is known that protein also may contribute to the production of fat in the body, though this may be through the formation of intermediary substances that are of the same nature as those formed from carbohydrate.

A question naturally arises as to how, if proteins, fats, and carbohydrates of food may all contribute to the production of body fat, the nature of the fat can still be to any significant degree characteristic of the species in which it is found. A partial explanation appears to be furnished by the work of Bloor and that of Frazer. Bloor finds that, when the fat of the food has been split to glycerol and fatty acids in the course of digestion and these digestion products are taken up and re-synthesized to fat in the intestinal wall, there may go into the re-synthesized fat not only the fatty acid radicles of the food fat but also fatty acid radicles formed in the body. These latter, entering into the constitution of the re-formed

fat, tend to give it some of the properties characteristic of the species while at the same time some of the characteristics of the food fat may be retained. Frazer (1946) finds that the digestive hydrolysis of fat carries some of it only to the mono-glyceride form, which fact, of course, works in the same direction emphasized by Bloor. Much study has been given to the effect of food upon body fat.

Limitation of space makes it necessary to confine ourselves here to the one phase of this work which is uppermost in the present discussion. Ellis and Isbell demonstrated anew the direct carrying over of some fatty acid radicles from food to body fat by the finding of linolenic acid in the lard of animals fed soybeans, and of arachidic acid in that of those fed peanuts. Anderson and Mendel developed a method of studying the influence of food upon the quality of the total body fat. Rats were fed a food-mixture in which 40 per cent of the calories were in the form of skimmed-milk powder and 60 per cent in the form of the food fat to be tested. The results as indicated by the iodine numbers * of the fats are summarized in Table 1. Here the influence of the food fat upon the body fat formed is clearly marked; but also there may here be seen the tendency of the body to modify the fat absorbed, so that the iodine numbers of the different body fats do not differ so *greatly* as do those of the corresponding food fats.

TABLE 1. INFLUENCE OF FOOD FAT UPON BODY FAT (Anderson and Mendel)

FOOD FAT (60% of Total Calories Fed)	IODINE NUMBER OF FOOD FAT	IODINE NUMBER OF BODY FAT
Soybean oil	132.3	122.5
Corn (maize) oil	124.3	114.2
Cottonseed oil	108.1	107.4
Peanut oil	102.4	98.4
Crisco	78.8	81.8
Lard	63.2	71.7
Butter fat	35.8	55.5
Coconut oil	7.7	35.3

* As noted above, *iodine number* is a technical term indicating what percentage of its own weight of iodine a fat will absorb.

Lipids as Body Constituents

From what has been stated above, fat is seen to be a form of reserve fuel to which any of the organic foodstuffs may contribute (see also Chapter 7). It is as reserve fuel that the large deposits of body fat are chiefly significant, but it should not be forgotten that even this "depot fat" may function as a protection to the body from mechanical injury or from too rapid a loss of heat when exposed to cold, and as a packing and support to the visceral organs, particularly the kidneys.

Lipoids

Lipoids are fat-like substances. The term does not usually include the fats and fatty acids themselves. The chief groups of lipoids are the phospholipins and the sterols, both of which are essential to the normal structure and functioning of the body.

Thus cell membranes are not simply walls of protein matter but are composed of both proteins and lipids of different kinds and in varying proportions, and the organic matter of protoplasm is to be regarded as regularly containing lipids as well as proteins.

The familiar subdivision of the phospholipids is into lecithins, cephalins, and sphingomyelins. Bloor makes a co-ordinate group of the glycolipids (cerebrosides, galactolipins).

The Macleans use the following classification:

I. Phospholipins (phospholipids, phosphatids)
   A. Monoaminomonophospholipins (atomic ratio, N : P : : 1 : 1)
      (*a*) Lecithins; (*b*) Cephalins (kephalins).
   B. Diaminomonophospholipins (N : P : : 2 : 1) Sphingomyelins.
II. Galactolipins (glycolipids, cerebrosides)
   (*a*) Phrenosin; (*b*) Kerasin; (*c*) Nervon.

The chief chemical relationships are as follows:

Lecithin has the molecular structure of a fat in which one of the fatty acid radicles is replaced by a substituted phosphoric acid which carries a choline radicle.

Cephalin has the same general structure but its nitrogenous radicle is different.

Sphingomyelin is not a glyceride (contains no glyceryl radicle and so is not a substituted fat), but is related to the fats, lecithins, and cephalins in that it contains a fatty acid radicle and a choline-phosphoric acid radicle. Each of these is linked to a central sphingosine radicle.

The cerebrosides (galactolipins, glycolipids) do not contain phosphorus but do contain fatty acid and nitrogen, the latter in the form of a sphingosine group which is thus a common feature of the sphingomyelin and cerebroside molecules.

Details of structure can readily be found in the textbooks, monographs, and reviews listed at the end of the chapter.

Phospholipids or **phosphatides** are widely distributed in living cells and doubtless essential to their structure and functions. The fatty matter (total lipids) of the active tissues of the body, as distinguished from that of the adipose tissue, seems to consist largely of phospholipids.

In all of the cases studied structurally by Levene, the lecithin molecule contains at least one unsaturated fatty acid; and Bloor and his coworkers (among others) emphasize the fact that in general there is a larger proportion of unsaturation in the fatty acids of the phosphatids than in those of the "neutral" or "depot" fats (the triglycerides such as those of the adipose tissue). It is largely for this reason that the phosphatids are regarded as possible intermediary steps in the metabolism of fatty acids. Whether or not they function thus in fat metabolism, the phospholipids are undoubtedly essential constituents of cell structure.

Those interested in phospholipid metabolism may well read the following four papers: *J. Biol. Chem.* **122,** 169–182; **123,** 587–593; **124,** 795–802; **126,** 493–500. *See also* the list at end of this chapter.

Sterols also are widely distributed in plant and animal cells and tissues. Doubtless many sterols are essential constituents of the organisms in which they occur. Some but not all sterols are among the vitamins D (Chapter 24).

Other lipoidal substances such as bile acids, sex hormones, and such things of primarily pathological interests as the carcinogenic hydrocarbons cannot be included within the scope of this book. Nor is space available here for the consideration of lipoids from the

viewpoint of their occurrence in vegetable tissues and their significance in the chemical processes of plant life.

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Chapter 4

GENERAL CHEMISTRY OF THE PROTEINS AND THEIR AMINO ACIDS

Carbohydrates and fats are the chief sources of energy for the activities of the body but not the chief constituents of which the active tissues are composed. Muscle tissue, for instance, contains but little carbohydrate, and often very little fat. The chief organic constituents of the muscles, and of protoplasm generally, are substances which contain nitrogen and sulfur in addition to carbon, hydrogen, and oxygen. Mulder, in 1838, described a nitrogenous material which he believed to be the fundamental constituent of tissue substances and gave it the name *protein,* derived from a Greek verb meaning "to take the first place." The term which Mulder thus introduced has been retained, and in the plural form, *proteins,* is now used as a group name to cover a large number of different but related nitrogenous organic compounds which are so prominent among the constituents of the tissues and in the cost of food that they may still be accorded some degree of preeminence in a study of the chemistry of food and nutrition.

Proteins are essential constituents of both plant and animal cells. There is no known life without them. Plants build their own proteins from inorganic materials obtained from the soil and air. Animals form the proteins characteristic of their own tissues, but in general they cannot build them up from simple inorganic substances such as suffice for the plants, and must depend upon the digestion products obtained from the proteins of their food. Since animals must have proteins for the construction and upkeep of their tissues, and since, broadly speaking, they cannot make their proteins except from the cleavage products of other proteins, it follows that proteins (or their cleavage products, the amino acids) are necessary ingredients of the food of all animals.

Chemical Nature and Physical Properties of Proteins

Generally speaking, the proteins of different kinds of tissue, and even of the corresponding tissues of different species, are not identical substances. The total number of different proteins occurring in nature must therefore be very great. Such natural proteins as have been sufficiently isolated and studied as to warrant description as chemical individuals have proven to be very complex substances and in no case has the chemical structure of a typical natural protein been *fully* determined. It has, however, been shown that the typical proteins are essentially anhydrides of the following amino acids: *

TABLE 2. CHEMICAL STRUCTURES OF THE AMINO ACIDS

Monoamino-monocarboxylic acids

Glycine, amino-acetic acid, $CH_2(NH_2) \cdot COOH$

Alanine, α-amino-propionic acid, $CH_3 \cdot CH(NH_2) \cdot COOH$

Valine, α-amino-β-methyl-*n*-butyric acid,

$$(CH_3)_2 : CH \cdot CH(NH_2) \cdot COOH$$

Leucine, α-amino-γ-methyl-*n*-valeric acid,

$$(CH_3)_2 : CH \cdot CH_2 \cdot CH(NH_2) \cdot COOH$$

Isoleucine, α-amino-β-methyl-*n*-valeric acid,

$$\begin{array}{l} CH_3 \cdot CH_2 \\ \qquad\quad \diagdown \\ \qquad\quad\ CH \cdot CH(NH_2) \cdot COOH \\ \qquad\quad \diagup \\ \qquad\quad CH_3 \end{array}$$

Norleucine, α-amino-*n*-caproic acid,

$$CH_3 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CH(NH_2) \cdot COOH$$

Phenylalanine, α-amino-β-phenyl-propionic acid,

$$C_6H_5CH_2 \cdot CH(NH_2) \cdot COOH$$

Tyrosine, α-amino-β-para-hydroxyphenyl-propionic acid,

$$C_6H_4(OH) \cdot CH_2 \cdot CH(NH_2) \cdot COOH$$

* In this first, introductory, listing of these amino acids the sequence is that of chemical relationship. Later listings in this book will, for convenience of reference, follow simply alphabetical sequence.

Serine, α-amino-β-hydroxy-propionic acid,

$$CH_2(OH) \cdot CH(NH_2) \cdot COOH$$

Threonine,[a] α-amino-β-hydroxy-*n*-butyric acid,

$$CH_3 \cdot CH(OH) \cdot CH(NH_2) \cdot COOH$$

Cystine (dicysteine), or di-(α-amino-β-thio-propionic acid),

$$\begin{array}{l} S \cdot CH_2 \cdot CH(NH_2) \cdot COOH \\ | \\ S \cdot CH_2 \cdot CH(NH_2) \cdot COOH \end{array}$$

Methionine, α-amino-γ-methylthio-*n*-butyric acid,

$$CH_3 \cdot S \cdot CH_2 \cdot CH_2 \cdot CH(NH_2) \cdot COOH$$

Monoamino-dicarboxylic acids

Aspartic acid, amino-succinic acid,

$$COOH \cdot CH_2 \cdot CH(NH_2) \cdot COOH$$

Glutamic (glutaminic) acid, α-amino-glutaric acid,

$$COOH \cdot CH_2 \cdot CH_2 \cdot CH(NH_2) \cdot COOH$$

Hydroxyglutamic acid, α-amino-β-hydroxy-glutaric acid,

$$COOH \cdot CH_2 \cdot CHOH \cdot CH(NH_2) \cdot COOH$$

Diamino-monocarboxylic acids

Arginine, α-amino-δ-guanidino-*n*-valeric acid,

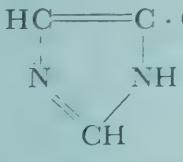
$$(H_2N)\overset{\overset{NH}{\vdots\vdots}}{C} \cdot NH \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CH(NH_2) \cdot COOH$$

Lysine, α, ϵ, diamino-*n*-caproic acid,

$$CH_2(NH_2) \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CH(NH_2) \cdot COOH$$

Heterocyclic amino acids

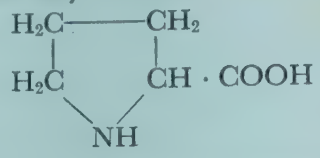
Histidine, α-amino-β-imidazole propionic acid,



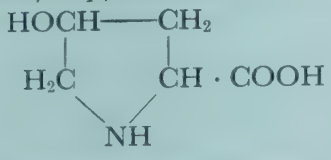
[a] To W. C. Rose and coworkers we are indebted for the discovery of this hitherto unknown substance, its actual (physical) isolation, its chemical identification, and the name indicative of its structural relationship to the tetrose sugar, threose.

TABLE 2. (*Continued*)

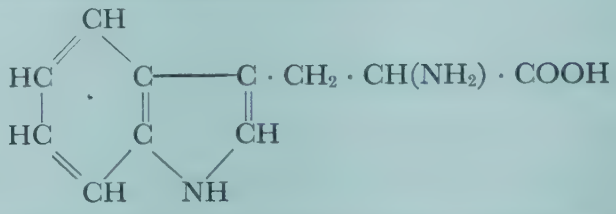
Proline, α-pyrrolidine-carboxylic acid,



Hydroxyproline, γ-hydroxy-α-pyrrolidine-carboxylic acid,



Tryptophane, α-amino-β-indole-propionic acid,



In addition to the amino acids which occur in proteins generally, there are a few others which have been reported only occasionally. Recently, too, the observation has been made that, besides amino acids, most proteins also contain a carbohydrate radicle which so persists through extensive purifications as to be regarded by some investigators as a component of the molecule. From certain proteins a carbohydrate complex consisting of two molecules of hexose and one molecule of hexosamine has been isolated. In a number of proteins, the hexose present has been identified as mannose, galactose, or a mixture of mannose and galactose.

It will be noted that the amino-acid constituents of the protein molecule differ much in structure among themselves. They are, however, all alpha-amino acids, *i.e.*, the amino group (or one of them if there be more than one) is attached to the carbon atom adjacent to the carboxyl. Proline and hydroxyproline may be regarded as only apparently exceptions to this statement, as in them the alpha-amino group is "condensed" with the delta-carbon.

The plan of indicating structural relationships by prefixing small capital D or L, has been so fully explained in biochemical publications as not to need further discussion here.

Several interesting and important differences among amino acids may be seen in the structural formulas listed above.

Amino Acids in the Protein Molecule

The linkage of the amino acid radicles in the protein molecule is chiefly (but probably not exclusively) through the carboxyl group of one amino acid reacting with the amino group of another. Thus two molecules of glycine, combined by elimination of one molecule of water, yield glycylglycine,

$$\begin{array}{l} H_2N—CH_2—C=O \\ \qquad\qquad\quad | \\ \qquad\qquad HN—CH_2—COOH, \end{array}$$

which is the simplest of the immense group of anhydrides of amino acids called *peptides.* Dipeptides contain two amino acid radicles; tripeptides contain three; and so on.

A certain analogy between the chief carbohydrates and proteins of the food may be noted. Starch on hydrolysis yields the polysaccharide dextrins, the disaccharide maltose, and finally (as end product) the monosaccharide glucose. Thus a typical native protein is hydrolyzed through proteoses, peptones, polypeptides, and di- or tri-peptides, to amino acids. In this sense, the amino acid bears the same general relation to the protein which glucose bears to starch; and just as the molecular weight of starch is very high and a single starch molecule yields a large number of monosaccharide molecules, so the molecular weight of the typical protein is very high and the protein molecule yields a large number of amino acid molecules. There is, however, this important difference: the molecules of monosaccharide resulting from complete hydrolysis of starch are all alike (glucose), whereas the complete hydroylsis of any typical protein yields several of the above-mentioned kinds of amino acids; in the case of most proteins, from twelve to twenty kinds. Investigations have shown, however, that, in the circumstances generally accepted as normal, only ten of these amino acids are independently "nutritionally essential" in the sense that each of these must be supplied by the food. These are listed and discussed separately in Table 5, in the next chapter, and they are also included in the list headed *Chemical Structures of the Amino Acids* above.

In Table 4 the percentages of these ten "essential" amino acids are given for each of 18 kinds of food protein.

The proportions in which a given amino acid radicle occurs in various proteins may be quite different. The four proteins, casein, gelatin, lactalbumin, and zein, yield from 0 to 25 per cent of glycine; from 1.8 to 10 per cent of alanine; from 3 to 7 per cent of valine; from 3 to 21 per cent of leucine. Of lysine, zein yields almost none, gelatin about 4 per cent, casein about 8 per cent, and lactalbumin about 11 per cent. Of tryptophane, zein and gelatin yield practically none, casein about 1.2 per cent, and lactalbumin about 1.7 per cent.

The ultimate composition of the proteins shows a general but not complete similarity. All contain carbon, hydrogen, oxygen, nitrogen, and sulfur; some contain phosphorus or iron also.

From the accompanying table of ultimate composition of twelve typical proteins, it will be seen that all these proteins contain 51 to 55 per cent carbon, about 7 per cent hydrogen, 20 to 23 per cent oxygen, 15.5 to 18.7 per cent nitrogen, 0.4 to 1.7 per cent sulfur. Other typical proteins thus far studied have shown ultimate composition within these same limits.

Similarity of elementary composition is entirely consistent with the belief that there may be an enormous number of chemical individuals among the proteins of nature.

TABLE 3. ELEMENTARY COMPOSITION OF SOME TYPICAL PROTEINS ACCORDING TO OSBORNE

	CARBON	HYDROGEN	NITROGEN	OXYGEN	SULFUR	IRON	PHOSPHORUS
	Per Cent	*Per Cent*	*Per Cent*	*Per Cent*	*Per Cent*	*Per Cent*	*Per Cent*
Casein	53.13	7.06	15.78	22.37	0.80	—	0.86
Edestin	51.50	7.02	18.69	21.91	0.88	—	—
Egg albumin	52.75	7.10	15.51	23.024	1.616	—	—
Gliadin	52.72	6.86	17.66	21.733	1.027	—	—
Lactalbumin	52.19	7.18	15.77	23.13	1.73	—	—
Legumin	51.72	6.95	18.04	22.905	0.385	—	—
Leucosin	53.02	6.84	16.80	22.06	1.28	—	—
Myosin	52.82	7.11	16.67	22.03	1.27	—	—
Ovovitellin	51.56	7.12	16.23	23.242	1.028	—	0.82
Oxyhemoglobin	54.64	7.09	17.38	20.165	0.39	0.335	—
Serum-globulin	52.71	7.01	15.85	23.32	1.11	—	—
Zein	55.23	7.26	16.13	20.78	0.60	—	—

Emil Fischer illustrated the vast number of isomers which may exist among polypeptides and proteins by pointing out that a synthetic 19-peptide obtained by linking 15 glycine and 4 leucine molecules is only one of 3876 possible isomers, without considering the tautomerism of the peptide linking. When more than two kinds of amino acids are involved, the possible number of isomers increases very rapidly. If a protein be imagined made up of 30 molecules of 18 different amino acids, one taken twice, one 3 times, another 3, one 4, one 5 times, and 13 taken once each, there would be ten to the 27th power (10^{27}) isomers even if there were no tautomerism of the peptide group and if the linking took place only in the simple way as with mono-amino-monocarboxylic acids.

It is easy to see that when one considers not only isomerism but the vast number of compounds of slightly different composition which can be obtained by varying the kinds and proportions of the amino acid radicles in the protein molecule, the possible number of different proteins of very similar elementary composition is practically unlimited.

Probable Molecular Weights of Proteins

The minimum molecular weight which would be consistent with the results of ultimate analysis would for many proteins be about 16,000 to 18,000. Thus, oxyhemoglobin contains only 0.335 to 0.34 per cent of iron, and since there must be at least one iron atom in the molecule, it is obvious from a simple proportion making use of the atomic weight of iron,

$$0.335 : 56 :: 100 : x,$$

that the molecular weight of hemoglobin must be in the neighborhood of 16,700 or a multiple of this.

Physico-chemical studies have indicated that the probable molecular weights of typical proteins are of the order of the multiples rather than of the analytically conceivable minimum just calculated. Thus, Cohn, Sørensen, and Svedberg, working independently and with different physico-chemical methods, all obtained data indicat-

ing for egg albumin a molecular weight of about 34,500. Svedberg, using methods for the direct determination of molecular weights, based upon the behavior of protein "solutions" (molecular dispersions) in an ultracentrifuge, found that the molecular weights of many typical proteins were approximately 1, 2, 3, or 6 times the molecular weight of egg albumin, while even much higher molecular weights were indicated in some cases.

The student will do well at this point to read carefully (whether as review or extension of the background which he brings to the present study) the chapters on proteins and amino acids in such modern textbooks as Conant's *Chemistry of Organic Compounds* and Thomas' *Colloid Chemistry.*

Further Chemical Properties of Proteins

In some cases proteins have been obtained in crystalline form. Generally speaking, the proteins may be regarded as typically colloidal substances. In view of the fact that the behavior of proteins in the tissues is largely dependent upon their colloidal character, it is of interest to bear in mind the very high molecular weights of the proteins as mentioned in the last paragraph. These enormously high molecular weights make it possible that in the case of a typical native protein the individual molecule is so large as to constitute a colloidal particle so that here the distinction between molecular and colloidal dispersions may disappear.

The amino acids of which the proteins are composed are, by virtue of their amino and carboxyl groups, amphoteric substances or ampholytes, capable of dissociating either as cations or as anions, depending upon the reaction (hydrogen ion activity) of the solution. For discussion of amphoteric nature in terms of the *zwitter-ion* theory, see Thomas' *Colloid Chemistry,* Cohn and Edsall's review, and other readings suggested at the end of this chapter.

As the proteins also are amphoteric substances that may dissociate either as cations or as anions, depending upon the hydrogen ion activity of the solution, it is evident that there must be some hydrogen ion activity at which the tendencies toward the acidic and basic

dissociations are equal. This is known as the *iso-electric point* because at this reaction the protein does not migrate in an electric field. This point or zone has been established for many proteins and other ampholytes. At this hydrogen ion activity, proteins are most easily precipitated by neutral salts or alcohol. This fact has been extensively used in work dealing with the purification of proteins and other amphoteric substances and in attempts to gain more knowledge of their chemical nature.

The proteins are insoluble in all of the usual solvents for fats (ether, acetone, chloroform, carbon disulfide, carbon tetrachloride, benzene, and petroleum distillate). They differ in their solubilities in water, salt solutions, and alcohol, and these differences play a considerable part in the present schemes of classification.

Recently, rapid methods of determining individual amino acids by their effects upon the growth or activity of selected species of microorganisms have been developed and widely used. The data thus obtained by Horn, Jones, and Blum (1950), are used in the present edition of this book.

Classification

There was formerly much confusion in the classification and terminology of the proteins, and some differences of usage will still be met in the literature. The majority of writers follow the recommendations made by the American Physiological Society and the American Society of Biological Chemists. The following is an outline of the classification thus recommended, to which have been added examples from among the food proteins:

I. SIMPLE PROTEINS

Protein substances which yield only amino acids or their derivatives on hydrolysis.*

(*a*) *Albumins.* Simple proteins soluble in pure water and coagulable by heat. Examples: egg albumin, serum albumin (blood), leucosin (wheat), legumelin (peas).

* This definition is not quite literally correct, since recent studies have shown the presence of traces of carbohydrate material in many of these so-called simple proteins.

TABLE 4. AMINO ACID DATA OF HORN, JONES, AND BLUM: U.S.D.A. MISC. PUBL. 696 (1950)

Grams Obtained from 100 Grams of Protein in Each Case

	ARGI-NINE	HISTI-DINE	ISO-LEUCINE	LEUCINE	LYSINE	METHIO-NINE	PHENYL-ALANINE	THREO-NINE	TRYPTO-PHANE	VALINE
Arachin	13.50	2.16	4.55	7.61	2.72	0.24	6.96	2.89	0.90	4.85
Casein	3.81	2.65	5.63	9.22	8.20	2.58	4.89	4.80	1.20	6.90
Conarachin	16.53	2.05	3.98	6.61	4.69	2.13	4.32	1.93	1.20	3.68
Gelatin	9.11	0.77	1.25	3.30	4.44	0.78	2.33	2.10	0.04	2.90
Glycinin	7.94	1.97	5.69	8.07	6.90	1.15	5.82	3.00	1.05	4.56
Lactalbumin	3.42	1.50	6.12	12.90	10.80	1.62	3.59	5.37	1.72	5.82
Ovalbumin	6.03	2.06	7.41	9.43	6.32	4.43	7.17	4.48	1.30	7.54
Ox muscle protein	7.87	1.98	6.50	9.37	10.00	2.75	4.58	5.80	1.30	5.85
Phaseolin (from beans)	5.97	2.24	6.69	10.50	7.20	1.12	8.04	4.16	0.50	6.00
Zein	1.95	0.76	5.03	21.10	0.21	1.41	7.30	2.62	0.03	3.98
Corn, whole, yellow	4.68	2.23	4.39	14.33	2.30	1.44	5.26	3.89	0.48	5.3
Egg, whole dried	8.50	2.30	8.73	12.55	7.88	2.74	7.31	5.91	1.71	6.99
Milk, dry skim	2.81	2.46	6.12	10.35	7.10	2.17	4.54	5.25	1.12	6.93
Oatmeal	7.31	2.20	4.40	7.76	2.96	1.04	4.58	3.60	1.04	5.22
Peanut flour	12.27	2.35	3.42	6.72	2.93	0.82	5.26	3.05	0.88	4.14
Soybean flour	7.79	2.38	4.98	7.49	7.16	0.99	4.76	4.55	1.12	4.90
Whole wheat	4.48	2.03	3.60	5.89	2.50	0.99	4.06	2.97	0.83	4.10
Yeast, dried brewers	4.70	1.62	4.93	6.76	7.28	1.10	3.72	5.28	0.94	5.28

(*b*) *Globulins.* Simple proteins insoluble in pure water, but soluble in neutral salt solutions. Examples: muscle globulin, serum globulin (blood), edestin (wheat, hemp seed, and other seeds), phaseolin (beans), legumin (beans and peas), vignin (cow peas), tuberin (potato), amandin (almonds), excelsin (Brazil nuts), arachin and conarachin (peanuts).

(*c*) *Glutelins.* Simple proteins insoluble in all neutral solvents, but readily soluble in very dilute acids and alkalies. The best-known and most important member of this group is the glutenin of wheat.

(*d*) *Alcohol soluble proteins (prolamins).* Simple proteins soluble in relatively strong alcohol (70–80 per cent) but insoluble in water, absolute alcohol, and other neutral solvents. Examples: gliadin (wheat), zein (maize), hordein (barley), kafirin (kafir corn).

(*e*) *Albuminoids.* These are the simple proteins characteristic of the skeletal structures of animals (for which reason they are also called scleroproteins) and also of the external protective tissues, such as the skin, hair, etc. Example: collagen, which when boiled with water yields gelatin.

(*f*) *Histones.* Soluble in water, and insoluble in very dilute ammonia, and in the absence of ammonium salts insoluble even in an excess of ammonia; yield precipitates with solutions of other proteins and a coagulum on heating which is easily soluble in very dilute acids. On hydrolysis they yield several amino acids, among which the basic ones predominate. The only members of this group which have any considerable importance as food are the thymus histone and the globin of hemoglobin.

(*g*) *Protamins.* These are simpler substances than the preceding groups, are soluble in water, not coagulable by heat, possess strong basic properties, and on hydrolysis yield a few amino acids, among which the basic amino acids greatly predominate. They are of no importance as food.

II. CONJUGATED PROTEINS

Substances which contain the protein molecule united to some other molecule or molecules otherwise than as a salt.

(*a*) *Nucleoproteins.* Compounds of one or more protein molecules with nucleic acid. Examples of the nucleic acids thus found united with proteins are thymo-nucleic acid (thymus gland), triticonucleic acid (wheat germ).

(*b*) *Glycoproteins.* Compounds of the protein molecule with a substance or substances containing a carbohydrate group other than a nucleic acid. Example: mucins.

(*c*) *Phosphoproteins.* Compounds in which the phosphorus is in organic union with the protein molecule otherwise than in a nucleic acid or lecithin. Examples: caseinogen (milk), ovovitellin (egg yolk).

(*d*) *Hemoglobins.* Compounds of the protein molecule with hematin or some similar substance. Example: hemoglobin of blood. (The redness of meat is also due chiefly to hemoglobin.)

(*e*) *Lecithoproteins.* Compounds of the protein molecule with lecithins or related substances.

III. DERIVED PROTEINS

1. *Primary protein derivatives.* Derivatives of the protein molecule apparently formed through hydrolytic changes which involve only slight alterations.

(*a*) *Proteans.* Insoluble products which apparently result from the incipient action of water, very dilute acids, or enzymes. Examples: casein (curdled milk), fibrin (coagulated blood).

(*b*) *Metaproteins.* Products of the further action of acids and alkalies whereby the molecule is sufficiently altered to form proteins soluble in very weak acids and alkalies, but insoluble in neutral solvents. This group includes the substances which have been called "acid proteins," "acid albumins," "syntonin," "alkali proteins," "alkali albumins," and "albuminates."

(*c*) *Coagulated proteins.* Insoluble products which result from (1) the action of heat on protein solutions, or (2) the action of alcohol on the protein. Example: cooked egg albumin, or egg albumin precipitated by means of alcohol.

2. *Secondary protein derivatives.* Products of the further hydrolytic cleavage of the protein molecule.

(*a*) *Proteoses.* Soluble in water, not coagulable by heat, precipitated by saturating their solutions with ammonium sulfate or zinc sulfate. The products commercially known as "peptones" consist largely of proteoses.

(*b*) *Peptones.* Soluble in water, not coagulable by heat, and not precipitated by saturating their solutions with ammonium sulfate or zinc sulfate. These represent a further stage of cleavage than the proteoses. (The term "peptone" was formerly applied to all digestion products not coagulated by boiling and is still popularly used in the same sense.)

(*c*) *Peptides.* Definitely characterized combinations of two or more amino acids. An anhydride of two amino acid radicles is called a "di-peptide"; one having three amino acid radicles, a "tri-peptide"; etc. Peptides result from the further hydrolytic cleavage of the peptones. As was mentioned above, many peptides have also been made in the laboratory by the linking together of amino acids.

Substances simpler than the peptones but containing several amino acid radicles are often called "poly-peptides."

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Chapter 5

NUTRITIONAL CHEMISTRY OF THE PROTEINS AND THEIR AMINO ACIDS

Except for a few cases presenting abnormalities or particular difficulties in digestion, it is now believed that the nutritive values of the food proteins depend essentially upon the kinds of amino acid radicles which they contain and the quantitative proportion of each. Hence we interpret nutritional differences among proteins essentially in terms of their amino-acid make-up.

Relation between Chemical Constitution of the Proteins and Their Food Values

The amino acids which result from the digestive or other hydrolysis of food proteins differ so widely in their chemical constitution (Chapter 4) as to make it improbable that they could function interchangeably in a quantitative sense in all the chemical reactions which the nutritional process involves. Yet it is easily conceivable that some of the amino acids may often be formed in the body from others.

Obviously all amino acids which are essential to the structure of body proteins are in this sense nutritionally essential substances; but the term *nutritionally essential* is often used to indicate that the substance in question *must be fed* (furnished by nutriment consumed).

The nature of the experimental evidence as to whether or not certain individual amino acids are nutritionally essential (or, to use the alternative term, *indispensable*) may now be considered.

Lysine and *tryptophane* may conveniently be discussed together, because of the evidence furnished for these two amino acids simultaneously by the work of Osborne and Mendel. In one series of their

experiments, young rats received food mixtures containing 18 per cent of casein, gliadin, or zein as the sole protein fed. It was found that casein sufficed not only for maintenance but also for excellent growth; gliadin sufficed for maintenance but for only very slight growth; zein did not suffice even for maintenance (Fig. 1). Gliadin

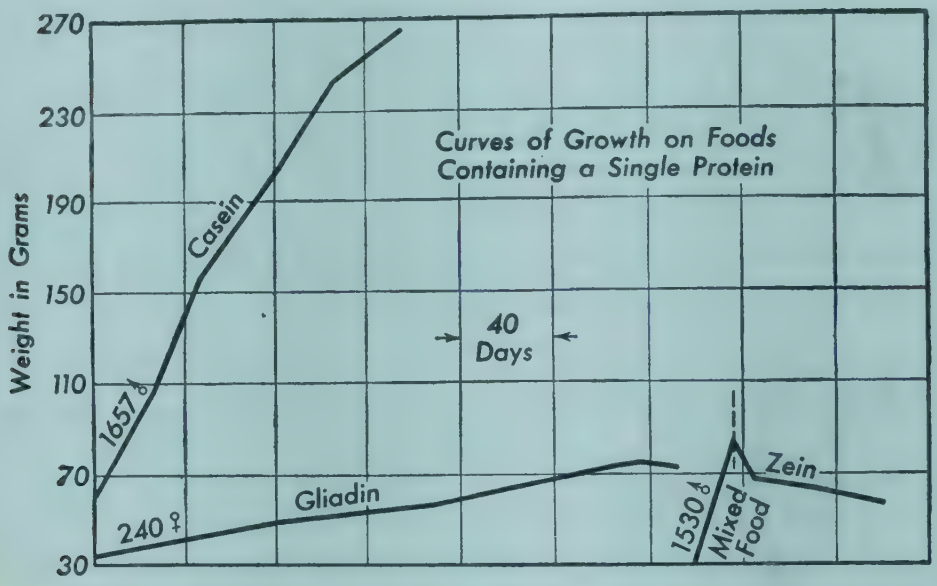

FIG. 1. Showing typical curves of growth of rats on diets otherwise similar and adequate but containing in each case only a single protein: casein, gliadin, or zein. (Courtesy of Dr. L. B. Mendel and the *Journal of the American Medical Association.*)

contains a small amount of lysine; and an additional small (and presumably constant) amount was introduced into both the gliadin and zein rations with the material used to supply the water-soluble vitamins. These facts were not always appreciated in the earlier interpretation of the results; but, when properly understood, do not materially detract from the value of the experiments, at least for the purpose of this discussion.

Supplementary experiments showed that more lysine was needed to permit normal growth on the gliadin diet; while the zein diet required addition of tryptophane also to make it adequate for growth (Fig. 2). Such evidence made it clear that lysine and tryptophane must each be furnished by the food.

W. C. Rose and coworkers find this to be true also for the maintenance of nitrogen equilibrium in man.

Cystine and *methionine* are also best studied together. Osborne and Mendel showed that, although casein fed as 18 per cent of the air-dry diet supported a normal rate of growth, when fed at a 9 per cent level, it permitted only about one-half as rapid growth. In this case the limiting factor was methionine plus cystine. For, the addition of cystine to the low-casein diet induced a normal rate of growth, which was immediately checked when the cystine was withdrawn and resumed when the cystine was again added to the

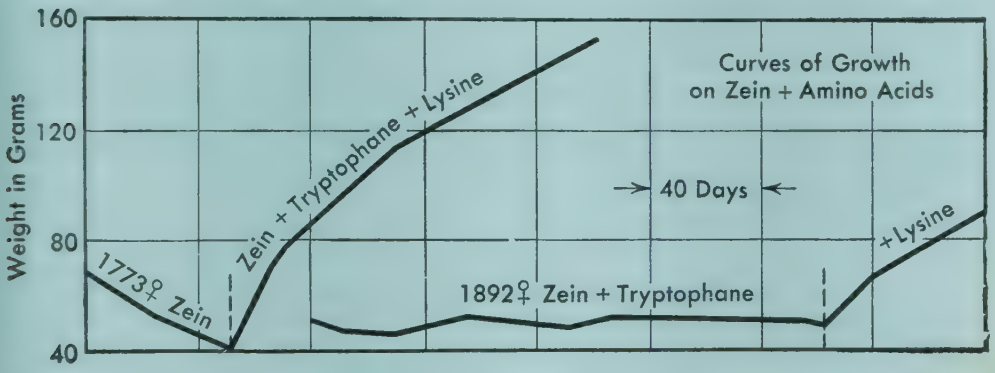

FIG. 2. Showing the effect of adding tryptophane or tryptophane and lysine to a diet containing zein as the sole protein. (Courtesy of Dr. L. B. Mendel and the *Journal of the American Medical Association.*)

ration. Later, the other sulfur-containing amino acid, methionine, was discovered in proteins, and found to be nutritionally essential. In further experiments by Rose and coworkers it has appeared that (both for growth in rats and for nitrogen equilibrium in man) methionine can function as precursor to supply the needed cystine; but that cystine does not perform all the functions of methionine.

Thus cystine is apparently dispensable and methionine indispensable in this sense; and as the term nutritionally essential has been commonly used in grouping the amino acids, methionine would be counted as nutritionally essential and cystine would not. Yet the cystine intake may actually be the determining factor in growth as in some of Osborne and Mendel's experiments. Hence, merely to memorize lists of amino acids as being or not being "dispensable,"

or "nutritionally essential" in the special sense so often used, may in some cases be misleading.

Which Amino Acids Are Indispensable Nutrients?

To keep in mind the special meanings given to certain terms in this connection, let us recall that W. C. Rose and his coworkers have demonstrated that a suitable mixture of amino acids is a nutritionally satisfactory substitute for protein even in the diet of rapidly growing experimental animals. He has shown also that if any one of ten so-called "nutritionally essential" or "indispensable" amino acids is lacking in the amino acid mixture, normal growth will not occur. But if all ten of these are provided in suitable amounts, the body can form from them the remaining amino acids which enter into the composition of its proteins. The ten amino acids that have appeared "indispensable," or "nutritionally essential" in this sense, are shown in Table 5.

TABLE 5. NUTRITIONALLY ESSENTIAL AMINO ACIDS AS EXPLAINED IN THE TEXT

(Arginine)	Methionine
(Histidine)	Phenylalanine
Isoleucine	Threonine
Leucine	Tryptophane
Lysine	Valine

Yet *arginine* and *histidine* seem dispensable in nitrogen equilibrium * experiments with adult man and so are placed in ( ) in the above list.

Presumably the difference is between the requirements of growth and of adult maintenance rather than a species difference, for the chemistry of the protein metabolism has been found to be very similar in rats and man.

Isoleucine, leucine, phenylalanine, and *valine* are apparently the typical simple cases of amino acids long known as constituents of body tissues, whose status as indispensable nutrients is now established by Rose's experimental feeding.

* Nitrogen balance and equilibrium are discussed in Chapters 8 and 11 beyond.

Lysine and *tryptophane* have, as noted above, been established as individually indispensable by both growth and nitrogen equilibrium experiments; and in these cases the empirical findings were clearly interpretable in terms of the unique structures of their respective molecules.

Threonine is unique in the fact that it remained undiscovered until Rose in his studies of these very problems of dispensability *versus* indispensability of individual amino acids found evidence of the presence (in certain proteins and their digestion products) of a nutritionally essential factor not previously known.

Methionine, with its relation to cystine which has been discussed briefly above, constitutes a case of a somewhat different kind. In later experiments than those above mentioned, Womack and Rose (1941) found cystine able to increase the growth rate when methionine is present in the diet in suboptimal amounts. And Rose and Wood (1941) showed definitely that the sulfur of methionine is utilized in the manufacture of cystine radicles which are built into the growing tissues.

"Essentiality" thus becomes a somewhat more complicated concept than it formerly appeared.

In view of recent discoveries regarding "the dynamic state of body constituents" (Schoenheimer, 1942) one may perhaps regard it as conceivable that exchanges of atoms and radicles may supply sufficient amounts of some of the amino acids for the slow turnover of adult maintenance, but not for the relatively rapid retention involved in the upbuilding of new tissue in the normally growing young animal.

Glycine, although an essential constituent of body tissue, need not be furnished by the food, for proteins which do not yield glycine on hydrolysis have been shown to be adequate when fed as sole protein of an experimental ration. It appears therefore that supplies of glycine fully adequate to meet all *normal* needs may be formed within the body itself; but the ability to form it is not unlimited, as Griffith has shown. When young rats are fed relatively large amounts of sodium benzoate, the benzoyl radicle of which they conjugate with glycine and excrete as hippuric acid, growth may cease because

this extra demand for glycine exceeds the ability of the body to form the amino acid. Under these conditions, the addition of extra glycine to the diet results in a resumption of growth, indicating that the cessation has been, in fact, due to a glycine deficiency thus experimentally produced.

Alanine is probably also formed in the body in ample amounts for normal needs. Feeding experiments with food supplies adequate in other respects and lacking alanine may not seem necessary because alanine is a constituent of all the food proteins which have thus far been studied in detail with reference to their amino acid make-up. There is, moreover, experimental evidence (Chapters 7 and 11) that the body can make alanine from lactic or from pyruvic acid, and as these latter are constantly being formed in the intermediary metabolism of carbohydrate, this would probably provide the body with a source of alanine whether it were furnished as such by the food proteins or not.

Some of the amino acids which are nutritionally essential in the sense that the body cannot synthesize them from substances normally occurring in the food, may be formed in metabolism from specific closely-related substances if these are supplied. For example, growth in animals stunted by histidine deficiency is resumed when imidazole lactic or imidazole pyruvic acid is fed; and similarly, indole lactic or indole pyruvic acid may successfully replace tryptophane in the diet of rats.

But the substituted lactic and pyruvic acids corresponding to the amino acid lysine have apparently no favorable effect in relieving lysine deficiency. Thus, presumably, the body can replace the α-hydroxyl or α-carbonyl group by an amino group to form histidine or tryptophane, but not to form lysine. Or, as regards histidine and tryptophane, the indispensable part is the unique heterocycle of each, and not the alanine sidechain, lactic and pyruvic acids being interconvertible with alanine (Chapters 7 and 11).

While much remains to be done in this field, the general plan of experimentation and interpretation initiated and carried to such an important series of correlations between chemical structure and nutritional function by Osborne and Mendel, and more recently by

W. C. Rose, H. B. Lewis, and their respective coworkers, already constitutes one of the most important advances in the development of the chemistry of food and nutrition.

Classification of Proteins as to Nutritional "Completeness"

To emphasize the differences between individual proteins they have sometimes been grouped as:

(1) "Complete": Maintaining life and providing for normal growth when used as a sole protein food. Examples of these are: Casein and lactalbumin from milk; ovalbumin and ovovitellin of egg; glycinin of soy bean; excelsin of Brazil nut; edestin, glutenin, and maize glutelin of the cereal grains.

(2) "Partially Incomplete": Maintaining life but not supporting normal growth. Gliadin of wheat is a well-demonstrated example of this class. Hordein of barley and the prolamin of rye are similar in nutritive value to gliadin.

(3) "Incomplete": Incapable either of maintaining life or of supporting growth, when fed as the sole protein. Zein of corn (maize) and gelatin are the conspicuous examples.

Any such grouping of the proteins, however, should be used with great care to insure an understanding of the quantitative aspects of the experimental data, if misconceptions are to be avoided. Edestin is a conspicuous example of a "complete" protein, having served as the sole protein food of a family of rats for three generations; but when the percentage of edestin in the food mixture was considerably reduced, results like those above described for gliadin were obtained—the diet did not support a normal rate of growth, but this could be secured by adding lysine to the food mixture. Similarly casein, when fed in greatly reduced proportion to the total food mixture, did not support normal growth; but growth became normal when cystine was added. Thus "complete" proteins may behave as "partially incomplete" when fed in seriously reduced proportion. It is also to be remembered that varying rates of growth in different species (not to mention other differences) make inadmissible any broad generalizations as to the proportion in which

any protein should be fed to species other than that with which its "completeness" or "incompleteness" has been demonstrated.

In 1916, Osborne and Mendel published quantitative measurements of the relative efficiency (for support of growth in young rats) of some of the "complete" proteins. The rate of gain obtained with 8 per cent of lactalbumin required 12 per cent of casein or 15 per cent of edestin; or, as they also state the results, "to produce the same gain in body weight 50 per cent more casein than lactalbumin was required, and of edestin nearly 90 per cent more."

Supplementary Relations between Proteins in Nutrition

In the feeding experiments shown in Fig. 1, each ration was planned to contain only a single protein. This is the ideal condition for the experimental comparison of isolated proteins, but is quite different from the ordinary conditions of practice, since our common ("native") protein foods all contain mixtures of proteins.

By feeding definite mixtures of pure proteins, Osborne and Mendel demonstrated clearly that proteins may supplement each other in nutrition. Thus zein is, as we have seen, inadequate as a sole protein food; lactalbumin is adequate when fed in sufficient quantity but when constituting only 4.5 per cent of the food mixture it supported only slow growth. Yet a food mixture containing 4.5 per cent of lactalbumin and 13.5 per cent of zein supported growth at a fully normal rate (Fig. 3). This shows that a relatively small amount of lactalbumin (one fourth of the total protein fed) sufficed to furnish the amino acid groups which the zein lacked. It shows also that zein, which when fed as a sole protein is insufficient even for maintenance, is able as a constituent of a proper food mixture to take part importantly in supplying the materials for growth as well as maintenance.

Thus zein, although inadequate for either maintenance or growth when isolated and fed alone, may nevertheless take an important part in both maintenance and growth when fed in a proper mixed diet. Moreover, it may not even be necessary to resort to a mixture of food materials in order to make good the deficiencies of the individual incomplete protein. Corn (maize) itself contains,

along with zein, an almost equal amount of another protein, maize glutelin, which Osborne and Mendel have shown to be capable of supporting a normal rate of growth.

Similarly, the other proteins of the wheat kernel supplement its gliadin by supplying more richly the lysine in which gliadin is

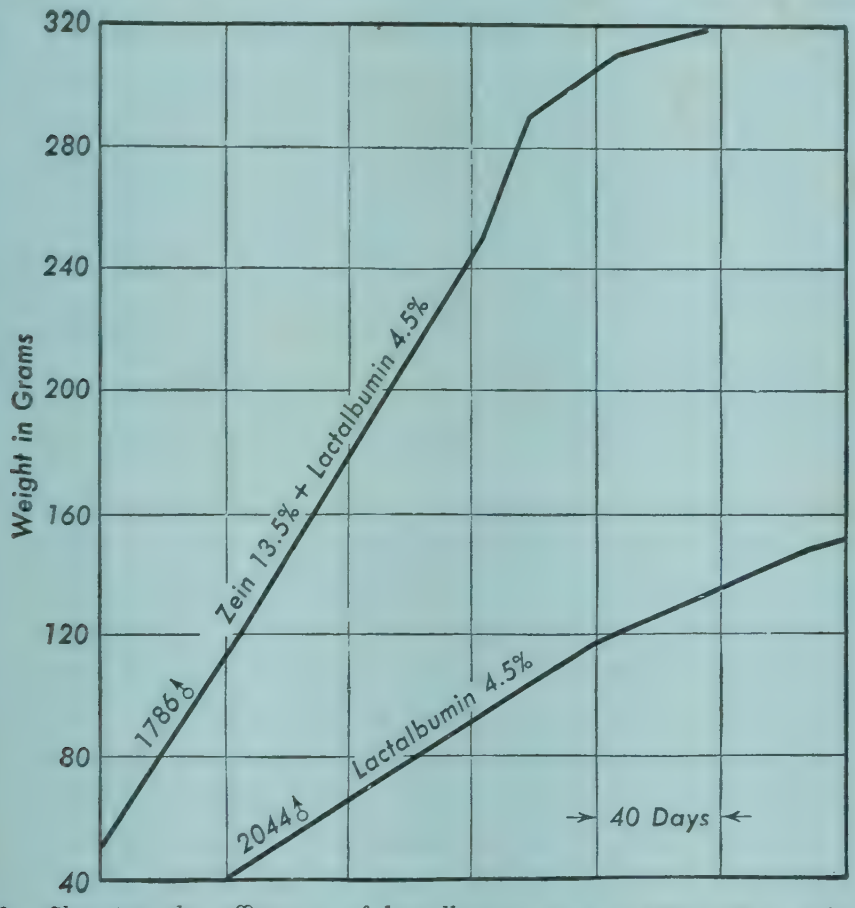

FIG. 3. Showing the efficiency of lactalbumin as a supplement to zein, and also that zein may take an important part in growth, although zein *alone* is inadequate either for growth or maintenance. (Courtesy of Dr. L. B. Mendel and the *Journal of the American Medical Association.*)

poor. Thus, as was strikingly shown in the findings of Osborne and Mendel and of Jones and coworkers, the natural protein mixture of whole wheat is much superior nutritionally to that of ordinary white flour. The term "enriched" as officially applied to flour and bread signifies the presence of added iron and vitamins (see Chapter 18) but does not imply anything as to the protein.

While it is plain that natural articles of food may contain proteins whose amino acid contents differ in such manner that these proteins supplement each other, it does not follow that the total protein mixture of one natural food is always as efficient as that of another.

Hart, McCollum, and their associates have shown that the natural protein mixture of milk is much more efficient than an equal weight of the mixed proteins of grain, both for the support of growth and as food for the production of milk in dairy cows. While it is always possible that in comparisons between natural food materials the results *may* be influenced by differences in unknown food constituents, yet in the cases here cited there is ample ground for confidence that the differing efficiencies ascribed to milk and grain proteins are mainly due to the same differences of chemical constitution (amino-acid make-up) to which are attributable the striking results obtained in the experiments previously cited in which isolated proteins were fed.

This is made more certain by the fact that subsequent experiments by Steenbock and Hart demonstrated that the protein mixture of a properly proportioned ration of grain and milk may show high nutritive efficiency, doubtless because milk proteins are rich in lysine and tryptophane which are the amino acids most effective in supplementing the proteins of the cereal grains.

Use of milk or non-fat milk solids, or of soybean or peanut flour, or wheat germ, in breadmaking is a recognized means (especially since the recent experiments of Jones and coworkers and of McCay) to supplement the proteins of the wheat flour and improve the nutritive efficiency of the protein-mixture of the resulting bread.

Woods, Beeson, and Bolin (1943) found methionine to be the limiting amino acid in the proteins of peas. With 0.3 per cent of added methionine (and correspondingly with any food that supplies the needed amount of methionine) the protein of peas becomes highly efficient in nutrition.

Thus with a knowledge of the nutritional chemistry of the proteins of various foods it becomes relatively easy so to utilize their supplementary relationships that even an inexpensive mixed diet shall be

safe from such shortages of individual amino acids as have been illustrated in the feeding experiments with isolated proteins. Also, it becomes important to reform the traditional habit of speaking of "animal protein" as if it alone were efficient in this connection, for we now know that several of the plant proteins are similarly effective.

Amino-Acid Derivatives as Enzymes, Hormones, or Other Nutritional Catalysts

Both by Willcock and Hopkins and by Osborne and Mendel it was suggested that nutritionally essential amino acids may function not merely as "building stones" to be used along with other amino acids in the growth and upkeep of tissue protein, but also in more individual and specific ways as precursors of hormones or other specifically essential substances in the body. And there is now abundant evidence that amino acids can function also as precursors of enzymes.

Glutathione

Investigation by Hopkins and others revealed the presence in active plant and animal tissues of an amino-acid derivative which he named glutathione. This is a tripeptide of glutamic acid, cysteine, and glycine. It appears to be an important factor in the oxidation and reduction reactions of the cell, probably by virtue of the reversible change between the sulfhydryl groups (—SH) of the *cysteine* radicles in the glutathione and the disulfide groups (—S—S—) of the *cystine* radicles in the oxidized product. When glutathione is oxidized, two molecules containing cysteine unite to form one molecule of the oxidized product containing cystine.

The mode of linkage of the three amino acid radicles in glutathione and its corresponding structural formula, based upon its reactions and properties, were reported independently by Nicolet and by Kendall, Mason, and McKenzie, in 1930.

Glutathione is readily soluble in water and gives an intense nitroprusside reaction which is characteristic of all active plant and ani-

mal tissues. This test consists, in general, of treating a suspension or an extract of tissue with potassium nitroprusside in the presence of ammonia. When the test is positive, a color resembling that of potassium permanganate is developed. It is probable that a reduction of the nitroprusside molecule takes place. Cysteine which contains the sulfhydryl group gives this test whereas its oxidation product, cystine, does not. Because of this, the reducing property of tissues was, even before the isolation of glutathione, more or less commonly ascribed to the sulfhydryl group, but until the work of Hopkins no experimental proof of this was available as cysteine was known to be too reactive to exist to an appreciable extent in the tissues. This property now appears to be largely due to the sulfhydryl groups of the glutathione molecule. As the oxidized form does not give the nitroprusside reaction, the oxidation and reduction of glutathione may readily be followed by means of this test.

Hopkins pointed out that "Equilibrium in the living cell would seem to be such that the greater part of the substance (glutathione) present exists in the reduced condition; but oxidation and reduction of the constituent sulphur groups are reversible processes in the tissues, and both forms may at any moment be present." ". . . factors are present in the tissues which promptly reduce the oxidized product whenever its concentration is raised above an equilibrium value."

Thus, while cysteine is too unstable and reactive to exist, to an appreciable extent, as such in the tissues, it is protected in glutathione, and furnishes the reactive sulfhydryl group of this important compound. Glutathione appears to be resistant to the proteolytic enzymes of the body and to exist to an appreciable extent in all active tissues.

Thyroxine

Thyroxine, an iodine-containing amino-acid derivative, formed in the thyroid gland and carried thence to the tissues of the body as a whole where it increases the rate of oxidation (energy metabolism), was isolated by Kendall in 1914.

In 1926, Harington published a method for greatly increasing the

yields of thyroxine obtainable from the thyroid gland and a much more comprehensive study of its properties than had previously been possible. This led to the synthesis of thyroxine which was accomplished by Harington and Barger in 1927. Its structure is, in part, that of a derivative of tyrosine. The original papers listed at the end of the chapter and the monograph by Kendall (1929) may be consulted for more detailed accounts of this important substance.

The relations of thyroxine to the rate of oxidation in the body, and to development and health, are discussed in Chapters 9 and 16 in connection with the study of the conditions affecting the energy metabolism and the functioning of the thyroid gland from the standpoint of the iodine metabolism, respectively.

Adrenine, Adrenaline, or Epinephrine

The compound called adrenine, adrenaline, or epinephrine is the active substance formed by the adrenal (suprarenal) glands. There are differences of opinion as to its part in the oxidation processes of the body, or in the mobilization of oxidizable material. Its influence upon the rate of oxidation in the body is less marked than that of thyroxine and also more rapid and transitory. Following an injection of adrenaline there is an almost instantaneous increase in the basal metabolic rate of energy used which rapidly reaches a maximum and then soon drops back to normal. The entire effect, on the basal metabolic rate (Chapters 8 and 9), of administration of epinephrine in the normal adult, is over in a few hours; whereas with thyroxine there is a well-defined delay, six to eight hours, before the basal metabolic rate is measurably increased, and the effects of a single injection upon the basal metabolic rate may still be observable for five or six weeks after the injection. Boothby has found that the extent of the increase in the rate of oxidation following the administration of epinephrine is dependent upon its concentration in the tissues. The other functions of epinephrine in the body extend beyond the scope of this book.

Epinephrine is related in structure to, and is probably a derivative of, tyrosine.

Insulin

Insulin is sometimes spoken of as being concerned in the burning of glucose in the body. This appears to be true in the sense that insulin helps to prepare glucose for the intermediary metabolism which may lead to oxidation; not in any sense which would imply that insulin is directly an oxidizing agent.

The most direct chemical evidence on the nature of the action of insulin appears to be that it causes a lowering of the concentration of glucose in the blood; and this is observed both in the diabetic and in the normal body. This furnished an experimental method by which the characteristic potency of insulin concentrates was measured and progress toward the isolation of the pure substance judged. In 1926, Abel obtained it in crystalline form.

Harington and Scott (1929), using a somewhat different method, found that their crystals were identical in appearance with the crystals isolated by Abel. The results of a study of four samples of crystals, two of which were prepared by the method of Abel and two by the method of Harington and Scott, showed a uniform potency and the chemical analyses agreed well.

Crystalline insulin gives the typical biuret, Millon, Pauly, ninhydrin, and xanthoproteic reactions, as well as specific color reactions for several individual amino acids. Thus insulin, like other typical proteins, is an amino-acid derivative. The molecular weight as indicated by the ultra-centrifuge technique is about 35,000. Jensen and Evans (1934) summarized convincing evidence that the hypoglycemic factor is identical with the crystalline protein, and not merely adsorbed or loosely bound to it. These authors also (1935) pointed out that "practically the whole of this protein molecule" may be accounted for in terms of a relatively few amino acids: tyrosine (12%); cystine (12%); glutamic acid (21%); leucine (30%); arginine (3%); histidine (8%); lysine (2%); phenylalanine and proline, too little for quantitative measurement, and that the most careful studies have failed to reveal any special non-amino-acid constituent (prosthetic group) which would explain the unique physiological action of crystalline insulin.

The hypoglycemic effect of the hormone appears therefore to depend, if not on the entire protein molecule, at least on some certain groupings of amino acids in such molecule.

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Chapter 6

ENZYMES AND DIGESTION

The carbohydrates, fats, and proteins as they exist in foods are in most cases not ready to be used by the body tissues in the exact form in which they are eaten, but must usually undergo more or less alteration in the digestive tract to fit them for absorption and assimilation. Insofar as the changes which the food undergoes in the alimentary tract are chemical, they are brought about mainly by the action of digestive enzymes.

Classifications and General Properties of Enzymes

The word *enzyme* (from the Greek "in yeast") was introduced by Kühne as a general designation for the substances formed in plants or animals which had previously been called *soluble* or *unorganized* ferments to distinguish them from fermentation organisms. As more and more of the activities previously regarded as characteristic of organisms have been found to be due to enzymes, the conception of enzyme action has broadened until now enzymes are commonly defined as "catalysts formed in plant or animal cells."

As most nutritional reactions are catalyzed in the body, we may, if we wish, call most of the body's organic chemistry enzyme chemistry.

Although each enzyme is generally supposed to be a definite chemical substance, the identification and classification of enzymes is based chiefly upon the changes which they bring about. Some of the better-known groups of enzymes are as follows:

1. The hydrolytic enzymes.
   a. Proteolytic or protein-splitting enzymes (proteases)
   b. Lipolytic or fat-splitting enzymes (lipases).

c. Amylolytic or starch-splitting enzymes (amylases).
d. Sugar-splitting enzymes (sucrase, maltase, lactase).

2. The coagulating enzymes, such as thrombin (thrombase), which assists in the clotting of blood; and rennin, which causes the clotting of milk.
3. The oxidizing enzymes (oxidases, dehydrogenases).
4. The reducing enzymes (reductases).

(3–4. Oxidation-reduction, and/or "tissue respiratory," catalysts.)

5. Those which, like the zymase of yeast, produce carbon dioxide without using free oxygen.
6. Enzymes causing the breakdown of a larger into a smaller molecule of the same composition, as in the production of lactic acid from glucose.
7. Enzymes causing chemical rearrangement without breaking down of larger into smaller molecules (mutases).

Terminology of the hydrolytic enzymes. Except insofar as some familiar enzymes continue to be known by their old established names (pepsin, rennin, trypsin, etc.), scientific usage now generally follows the suggestion of Duclaux that each hydrolytic enzyme be designated by a name indicating the kind of substance on which it acts, together with the suffix *ase*. Thus starch-splitting enzymes are called *amylases;* fat-splitting enzymes, *lipases;* protein-splitting enzymes, *proteases*. The name showing the kind of change brought about or catalyzed by the enzyme is often preceded by an adjective to indicate its source: e.g., *salivary amylase* (ptyalin), *pancreatic amylase* (amylopsin). Such designation does not necessarily imply that the amylase found in the saliva either is or is not the same substance as the amylase of the pancreatic juice. Whether enzymes from various sources which have the same specific catalytic activity are identical chemically has been investigated in the case of several highly purified enzymes. From recent work it appears, for instance, that the crystalline pepsins from cattle and from swine are different substances; and that so are the crystalline trypsins from these two species. It has also been shown that pancreatic amylase differs from malt amylases, and that these latter differ also from one another.

In discussions of enzyme action the substance on which the enzyme acts is commonly called the *substrate*.

Within the cell producing it, an enzyme often exists in an inactive

form known as the *zymogen,* or antecedent of the active enzyme. The zymogen may be stored in the cell in the form of material which is converted into active enzyme at the time of secretion, or the secretion may be poured out with the zymogen not yet completely changed to active enzyme, or sometimes in a form which requires the presence of some other substance in order to render it active. In this case the latter substance is said to *activate* the enzyme. Activation may also mean a simple increase of activity.

Prominent among chemical studies of enzymes are: (1) attempts to ascertain what factors influence, or are necessary for, their activity; and (2) attempts to concentrate, purify, or isolate the active material. The second of these necessarily depends largely upon the first because enzymes are known only by their characteristic catalytic activities and any thoroughly satisfactory attempt to study them must involve quantitative measurements of their action. Solutions containing enzymes may readily be obtained from plant or animal tissues by extraction with suitable solvents. Thus, for example, the enzymes of the pancreas may be obtained in solution by grinding and extracting the gland. Such extracts contain many substances which were naturally present with the enzyme, some of which may be necessary for its stability in solution or for its normal activity. These necessary substances must be supplied in proper concentrations when the purified material is examined for activity. Among the many factors which are known to influence enzyme action markedly is the hydrogen ion activity of the enzyme-substrate system. For most enzymes this must be adjusted within rather narrow limits if optimal results are to be obtained; and in some cases even to obtain any positive result. This hydrogen ion activity is commonly referred to in terms of pH, which is the symbol introduced by Sørensen to represent the negative logarithm of the hydrogen ion concentration (activity), or

$$\mathrm{pH} = \log \frac{1}{[\mathrm{H}^+]}.$$

As the hydrogen ion activity most favorable to the action of an enzyme depends upon many interrelated factors, it should not be

stated dogmatically. Remembering this, it is nevertheless of interest to note the values which have been reported as being most favorable for the action of a few of the typical enzymes. Thus, invertase (sucrase) from yeast has been reported to be most active in solutions of about pH 4.4 (Nelson), while that from the intestinal digestive juice has been found to be most active in solutions of about pH 7.0. The amylases of barley malt are most active in solutions of about pH 4.5, while that from the pancreas exerts its maximum activity in solutions of about pH 7.0 to 7.2.

With some enzymes the optimal pH is different for different substrates. Thus pepsin acting on gelatin shows a pH optimum of about 2.4, whereas when acting on casein the optimum is about pH 1.8 (Northrop, 1922). Here, and also in the case of trypsin, the changes in activity with pH seem to be related to the state of ionization of the substrate, the acid salt of the protein being most readily attacked by pepsin, the alkali salt by trypsin (Northrop, 1922). An interesting interpretation of the effect of hydrogen ion activity on enzyme activity was suggested by Michaelis for invertase (sucrase). He pointed out that the curve relating reaction velocity to pH is very similar to that relating the concentration of the unionized fraction of an amphoteric electrolyte to pH, with the optimum hydrogen ion activity for the enzyme corresponding to the isoelectric point of the ampholyte. He suggested that invertase is in fact an ampholyte of which only the unionized form is catalytically active. A similar relation was found with several other enzymes, while in some cases the active form of the enzyme appeared to be ionic. Using a direct and ingenious method, Northrop (1924, 1925) showed that trypsin behaves as a univalent positive ion between pH 2 and 10, and pepsin as a univalent negative ion between pH 1 and 7. With these enzymes, the changes in activity with pH seem to be related to the state of ionization of the substrate.

Activities of Some Digestive Enzymes

That the typical digestive enzymes are very pronounced catalysts may be judged from the relatively large amounts of material which

they are capable of digesting under favorable conditions. Thus, pancreatic amylase when highly purified tends (as stated above) to lose its activity rather rapidly in solution, yet in 30 minutes at 40° C., one part of purified pancreatic amylase hydrolyzed about 20,000 parts of starch and formed about 10,000 parts of maltose (along with about an equal amount of dextrin). And in longer experiments it hydrolyzed 4,000,000 times its weight of starch with the formation of 2,800,000 times its weight of maltose before it had all become inactivated. This marked enzymic activity was exhibited by the preparation at a dilution of 1 : 10,000,000 parts of water. The most delicate tests for proteins are not valid at dilutions greater than about 1 : 100,000. The purified pancreatic amylase preparation reacted like typical protein to the usual protein tests, but its own enzymic activity constituted a test for its presence which was 100 times more delicate. Thus the failure of protein reactions in solutions enzymically active does not show that no protein is present or that the enzyme is of other than protein nature in its chemical composition, although this negative conclusion was erroneously drawn by some investigators and repeated by many writers.

At this point, one may well study the accompanying summary of the occurrence and action of the chief *digestive* enzymes (Table 6).

It may perhaps be asked why, if enzymes act by catalysis, there should be any limit to the amount of substrate which the enzyme can hydrolyze. One reason that enzymes cannot hydrolyze infinite amounts of substrate is that they are themselves unstable organic substances which necessarily undergo decomposition, especially when in solution. In most cases, the purer the enzyme the more rapidly its solutions lose their activity. Another reason that an enzyme does not continue to hydrolyze substrate indefinitely is that the reaction is progressively retarded by the accumulation of the products formed.

The activity of certain enzymes may be stopped, even when all other conditions are favorable, by the accumulation of the product of the reaction; and, in certain circumstances, the action of the enzyme may be reversed so as to accelerate a change in the opposite direction to that in which it ordinarily acts.

TABLE 6. SUMMARY OF CHIEF DIGESTIVE ENZYMES

	Enzymes	*Secreted by*	*Action*
Act on Carbohydrates	Ptyalin (salivary amylase)	Salivary glands	Converts starch to maltose
	Amylopsin (pancreatic amylase)	Pancreas	Converts starch to maltose
	Invertase (sucrase)	Intestinal mucosa	Converts sucrose to glucose and fructose
	Maltase	Intestinal mucosa	Converts maltose to glucose
	Lactase	Intestinal mucosa	Converts lactose to glucose and galactose
Act on Fats	Lipases	Gastric mucosa and pancreas	Split fats to fatty acids and glycerol
Act on Proteins	Pepsin	Gastric mucosa	Splits proteins to proteoses and peptones [a]
	"Trypsin" (actually a group of enzymes)	Pancreas	Splits proteins to proteoses, peptones, polypeptides and amino acids [b]
	"Erepsin" (actually a group of enzymes)	Intestinal mucosa	Splits peptones to amino acids and ammonia [c]

[a] When pepsin is allowed to act for many hours, lower protein breakdown products such as poly- and dipeptides and even free amino acids may be liberated, but under normal conditions of digestion *in vivo* the hydrolysis of protein *by pepsin* is not so extensive.

[b] Northrop has isolated from pancreas two crystalline proteolytic enzymes which he has called *trypsin* and *chymotrypsin*, both of which differ from earlier preparations of "trypsin."

[c] The catalytic reactions attributed to "erepsin" have more recently been attributed to a number of different enzymes occurring together.

The Chemical Nature of Some Typical Enzymes

In 1902 Pekelharing prepared a specimen of purified pepsin, which contained carbon, hydrogen, nitrogen, and sulfur in proportions within the range of variation found among ordinary proteins. It also behaved like ordinary proteins in the xanthoproteic test and Millon reaction and in showing the presence of the tryptophane group.

Dezani, in 1910, carried forward the work upon the chemical nature of pepsin by preparing what was believed to be a substantial duplicate of Pekelharing's product and submitting this to hydrolysis, followed by search for individual hydrolytic products according to the methods which had recently been developed in the study of the structure of the proteins. He demonstrated the presence of leucine, tyrosine, arginine, histidine, and lysine and also found evidence of other amino acids which the limitations of his material and methods did not permit him to identify.

Northrop (1929, 1930) has further established the protein nature of pepsin by physico-chemical study of crystalline pepsin. Thus pepsin as prepared by several investigators is a nitrogenous material not identical with any other known substance but complying with the criteria of our present conception of a protein in elementary composition, in color reactions, and especially in yielding the familiar amino acids upon hydrolysis.

Even earlier than Pekelharing's work on pepsin, Osborne (1895) had published an investigation of the chemical nature of diastase (malt amylase), which may be regarded as marking the beginning of our modern knowledge in this field, though recognition followed so tardily that its importance is perhaps not adequately reflected in the literature of the subject. From Osborne's work it appeared that the enzymic activity is a property of a definite fraction of the protein material of the malt, or in other words that the enzyme is protein in its chemical nature.

Following the confirmation of Osborne's work on malt amylase, pancreatic amylase also has been studied by modern methods with reference to its chemical nature.

In an investigation * in which attempts at purification were guided and their success largely judged by quantitative determinations of the enzymic activity of the products, there was developed a method of purification which in numerous independent experiments yielded material that was not only extraordinarily active in the hydrolysis of starch but was essentially uniform both in enzymic activity and

* *Journal of the American Chemical Society* 33, page 1195; 34, page 1104; 35, page 1790; 48, page 2947. *Journal of Biological Chemistry* 88, page 295.

in chemical nature. Such a result in itself indicates strongly that this material represents at least some approximation to an actual isolation of the enzyme, and this finding has been confirmed by several independent methods of investigation, including the crystallization of the purified substance. These preparations show the composition and color reactions of typical proteins and, like Osborne's malt amylase, the material when heated in water solution yields an albumin coagulum and a proteose or peptone which remains in solution. Moreover, on hydrolysis the material yields the same groups of amino acids which are yielded by typical proteins such as casein, which it also resembles in elementary composition.

Although the amylase of the saliva has not been so extensively studied, it appears, from the work of Tauber and Kleiner (1934) to be also of protein nature, since ptyalin preparations lost their enzymic activity when acted upon by proteases.

Highly purified preparations of pancreatic lipase give the typical protein color tests, and in solubility and precipitation reactions this enzyme has the characteristics of a globulin (Glick and King, 1933).

In the case of the typical enzyme malt amylase, the enzymic activity has been found to be so inseparable from the particular protein in which it resides as to migrate with it under the influence of the electric current in either direction according to the hydrogen ion activity of the solution. Thus this enzyme shows, like other protein substances, a characteristic isoelectric point. For further discussion of the significance of this observation, both as added evidence of the protein nature of the enzyme and as throwing light upon the conditions which influence the enzymic activity, the reader is referred to the original paper (Sherman, Thomas, and Caldwell, 1924).

There is also much indirect evidence of several independent kinds supporting the view that typical enzymes are of protein nature or contain protein as a constituent, in addition to the large amount of evidence derived from investigations upon the purification of typical enzymes and study of the chemical nature of the purified preparations. And the enzyme activity has repeatedly been found to

depend upon keeping the protein intact, activity being lost when the enzyme solution is subjected to treatment which coagulates, hydrolyzes, or otherwise induces chemical change in the protein matter.

Especial mention may also be made of the effect of added amino acids in diminishing the rate of hydrolytic inactivation; and of the quantitative finding that antiseptics which act chemically upon proteins are very much more destructive of the enzyme than are antiseptics of the lipoid-dissolving type. These independent lines of evidence are especially convincing because they link inactivation of the enzyme so closely and definitely with chemical change of the protein matter of the purified enzyme preparation; or, conversely, enzyme activity with intact protein.

Thus there is now abundant reason to accept the conclusion that the best-studied enzymes of the digestive tract are of protein or protein-like nature, and that food protein furnishes amino acids to serve as material for body enzymes as well as for body tissue in the more familiar sense. (To avoid even temporary misapprehension, the existence of tissue enzymes and coenzymes containing vitamin constituents may be mentioned here, though the study of them belongs with later chapters.)

Other digestive enzymes. Obviously, the above discussion does not include all enzymes that function in the digestion of food. Others, such as phosphatases, lecithinases, nucleotidases, may be studied in the more detailed literature referred to at the end of this chapter and also of Chapter 7.

With this brief sketch of the digestive enzymes, the adequate discussion of which would require a volume in itself, we may now pass to a review of the digestive process: the course of the food through the human alimentary tract, and the mechanical and chemical treatment to which it is subjected.

Salivary and Gastric Digestion

The muscular contractions of the empty stomach which give us the sensation of hunger may be regarded as an indication that the

stomach is in a state of readiness to receive food. There is experimental evidence that the stomach digests more expeditiously the food which is "eaten with hunger."

The description of the digestive process which follows presupposes that the food is eaten under favorable conditions and received by a digestive tract which has been permitted to form good and regular habits.

The eating of food induces a flow of saliva from great numbers of minute glands in the lining membrane of the mouth and from the three pairs of large salivary glands. That saliva is secreted in response to psychic as well as chemical stimulation is shown by the fact that actual contact with the food is not necessary, since secretion may be started by the sight or odor or even the thought of food. Mixed human saliva has usually a faintly alkaline reaction and always contains a starch-digesting enzyme (salivary amylase, ptyalin), although its amylolytic activity appears to vary considerably with individuals and with the same individual at different times of the day.

As the food comes in contact with saliva, the digestion of starch and dextrin under the influence of the salivary amylase begins at once; but, as mastication is an entirely voluntary act, the thoroughness with which the food becomes mixed with saliva is subject to wide variations.

Usually the food stays too short a time in the mouth for the starch to be acted upon *there* to any great extent, and formerly it was supposed that salivary digestion must cease almost as soon as the food reaches the stomach because the acidity of the gastric juice is unfavorable to the action of the amylase. The view has now changed as the result of a number of investigations, among which those of W. B. Cannon and of Carlson are of especial interest.

When a small amount of an inert metallic compound such as bismuth subnitrate is mixed with the food, it becomes possible to photograph the food-mass within the body by means of Roentgen rays (X-rays). By the use of this method Cannon carried out an extended series of observations of the movements of the stomach and intestines during digestion, upon the results of which the state-

ments concerning the mechanism of digestion in this chapter are chiefly based.

It is important to distinguish between (*a*) the elastic and relatively inactive *cardiac* region of the stomach, and (*b*) its muscular *pyloric* region—the part nearer the outlet to the small intestine.

Cannon's observations, confirmed by those of other investigators, show that the vigorous muscular movements described by Beaumont, which generally begin 20 to 30 minutes after the beginning of a meal, occur only in the middle and pyloric portion of the stomach, while the cardiac region or fundus, which serves as a reservoir for most of the food as it enters the stomach, is not actively concerned in these movements and does not rapidly mix its contents with the gastric juice.

That there is no general circulation and mixing of the entire stomach contents during or immediately following a meal is further shown by the experiments of Grützner, who fed rats with foods of

FIG. 4. Section of frozen stomach of rat during digestion to show the stratification of food given in three successive portions differently colored. (From Howell's *Textbook of Physiology*, by permission of the W. B. Saunders Company.)

different colors and, on killing and freezing the animals and examining the stomach contents, found that the portions which had been eaten successively were arranged in definite strata. The food which had been first eaten lay next to the walls of the stomach and filled the pyloric region, while the succeeding portions were arranged regularly in the interior in a concentric fashion. (Fig. 4). In describing this result Howell wrote: "Such an arrangement of the food is more readily understood when one recalls that the stomach has never any empty space within; its cavity is only as large as its contents, so that the first portion of food eaten entirely fills it, and successive portions find the wall layer occupied and are therefore received into the interior."

The character of the gastric juice secreted in different parts of the stomach varies considerably, especially as regards its acidity. In the middle region the secretion is rich in acid, while at both the cardiac and the pyloric ends, the *parietal cells* (formerly called the *border cells* or *cover cells*), from which the secretion of the acid appears to take place, are few in number or entirely lacking, and the juice secreted in these regions may be neutral or, according to Howell, even slightly alkaline.

The nature and extent of the muscular movements also vary greatly in the different regions of the stomach. The peristaltic waves of muscular constriction which bring about the thorough mixing of the food with the gastric juice begin in the middle region and travel toward the pylorus.

The food in the cardiac end of the stomach is not much moved by peristalsis, and so comes only slowly into contact with the gastric juice. And since the juice secreted here contains little if any free acid, a large part of the food mass remains for some time (variously estimated at from 30 minutes to 2 hours or more) in approximately the same neutral or faintly alkaline condition in which it was swallowed, and salivary digestion continues in this part of the stomach. Thus, if the food has been thoroughly chewed and well mixed with saliva before swallowing, much if not most of its starch may be converted into dextrin and maltose in the cardiac region of the stomach before the activity of the ptyalin (salivary amylase) is stopped by contact with the acid of the gastric juice.

The fundus, however, is not entirely inactive; rather it functions as a sort of elastic pouch which is distended by, and slowly contracts upon, the food mass, thus gradually tending to move it (and particularly its more fluid portions) into the pyloric region.

At intervals the pylorus opens and permits passage of chyme (the fluid or semi-fluid portion of food material mixed with, and partly digested by, the saliva and the gastric juice) into the small intestine. (If fuller discussion on this point is desired, see Murlin's review listed among references at the end of the chapter.)

As digestion proceeds, the pylorus opens more frequently and the stomach tends to empty itself more and more freely, until finally the

pylorus may open to allow the passage of particles which have not been acted upon by the gastric juice.

Whether the stomach will thus completely empty itself of one meal before the eating of the next will depend both upon the length of the interval and the amount and character of the food which was eaten. Small test meals may disappear in from 1 to 3 hours, but meals approximating one third of the day's food may not disappear entirely from the stomach during 6 hours.

Ordinarily, when each is fed separately, protein food stays longer in the stomach than carbohydrate; fat longer than protein. Mixtures of fat and protein leave the stomach more slowly than either when fed alone. In general the softer or more fluid the fat of the food, the more rapidly it will leave the stomach. Also, emulsified fats tend to pass on more promptly than fat of the same kind taken in larger masses.

The most important constituents of gastric juice are free hydrochloric acid and pepsin. While other acids may be found in *stomach contents*, the acidity of *gastric juice* appears to be due almost entirely to hydrochloric acid, which Carlson has found to constitute an average of 0.40 to 0.50 per cent of normal human gastric juice as secreted. This acid has an antiseptic action on the stomach contents.

When, through any cause, the acidity of the gastric juice is abnormally decreased, the numbers of bacteria in the stomach contents may increase greatly. Also the acidity of the chyme as it passes the pylorus has an important influence upon the secretion of the pancreatic juice.

Carlson has distinguished between three types of normal gastric secretion. There is a slight but continuous secretion of juice in the empty stomach. The flow is augmented by both psychical and chemical stimulation. The psychic secretion (of appetite juice) is brought about to some extent by the sight, odor, or memory of food but principally by the acts of tasting and chewing it. Since the continuous secretion is sufficient to initiate gastric digestion, the psychic secretion is not indispensable; and at best it usually ceases within 15 to 20 minutes after the completion of mastication. The chemical

stimulation arising within the stomach itself provides for the continuance of gastric digestion, and is thus probably more important than either the "continuous" or the "psychic" secretion. This third type of secretion is brought about by the presence of food in the stomach and also by the stimulating effect of certain substances, including water and dilute acids, which act through some local secretory mechanism in the walls of the stomach and duodenum.

Under normal conditions, the amount of nutritive material absorbed from the stomach is insignificant as compared with the amount absorbed from the intestine. Most of the food eaten is passed from the stomach into the intestine in the form of chyme, having been more or less liquefied and acidulated by its thorough mixing with the gastric juice in the middle and pyloric regions of the stomach.

The stomach therefore has several functions. It serves (1) as a storage reservoir receiving food in relatively large quantities, say three times a day, passing it on to the intestine in small portions at frequent intervals, (2) as a place for the continuation of the salivary digestion of starch, and (3) for the beginning of the digestion of proteins and perhaps fats, and finally (4) as a disinfecting station of somewhat doubtful and variable value since the food is subjected to the acidity of the gastric juice for a relatively short time in the pyloric region, and the degree of contact of acid with bacteria must depend largely upon the size of the food particles at this stage of digestion.

Intestinal Digestion

Digestion in the small intestine. When the pylorus opens, food, now reduced to liquid chyme, is projected into the upper part of the small intestine, where it usually lies for some time in the curve of the duodenum, until several additions have been made to it from the stomach. While the food rests here the bile and pancreatic juice are poured out upon it, and here also, as well as in other parts of the small intestine, a considerable amount of intestinal digestive juice is secreted by the glands of the lining membrane and mixed

with the intestinal contents. While for the purposes of description the pancreatic and intestinal juices and the bile may be discussed separately, it is to be remembered that in normal digestion they always act together. Cannon's observations showed that after a certain amount of food and digestive juices has accumulated as just described in the first loop of the small intestine, the mass all at once becomes segmented by constrictions of the intestinal walls, and the segmentation is repeated rhythmically for several minutes, so that the individual portions are subjected to relatively extensive and energetic to-and-fro movement, which is doubtless very important in facilitating the emulsification of fat. Other effects of the muscular constrictions which cause the segmentation are (1) a further mixing of food and digestive juices, (2) the bringing of the digested food into contact with the absorbing membrane, (3) the emptying of the venous and lymphatic vesicles in the membrane, the material which they have absorbed being forced into the veins and lymph vessels by the compression of the intestinal wall.

The fluid food mass which the stomach pours into the duodenum normally contains a small amount of free hydrochloric acid besides a larger amount combined with protein. The pylorus having closed, the bile, the pancreatic juice, and the intestinal juice (all being alkaline) act together to neutralize the acids present.

In man the main duct of the pancreas and the bile duct unite and empty into the small intestine about 8 to 10 cm. (3 to 4 inches) below the pylorus. *The pancreatic juice* is a clear, slightly alkaline liquid containing (or furnishing through their zymogens) not less than three enzymes which play important parts in the digestion of proteins, fats, and carbohydrates respectively.

The outflow of the pancreatic juice begins at once when any of the acid stomach contents passes through the pylorus, and has been shown by Bayliss and Starling to be due to a definite chemical substance, *secretin*, a hormone produced as the result of the action of the acid upon some constituent of the intestinal mucous membrane. Secretin is absorbed and carried by the blood to the pancreas and there stimulates the flow of pancreatic juice.

The bile in the intestinal contents greatly increases the solubility

of the fatty acids, while at the same time it diminishes the surface tension between watery and oily fluids, thus breaking up the oil droplets and permitting the lipase of the digestive juice to come into more effective contact with the fat of the food. More importantly, it has been shown that bile acids greatly facilitate the absorption of the fatty acids liberated in the digestion of fat. (A review by Verzar is cited among the references at the end of the chapter.) This behavior of the bile acids is explained by *in vitro* studies in which it was demonstrated that taurocholic and glycocholic acids possess the ability to bring into aqueous solution fatty acids, and, to a less marked extent, neutral fat.

According to Starling, the rapid flow of bile during intestinal digestion is due not only to the pouring out of what was previously stored in the gall bladder, but also to an increased rate of secretion to which the liver is stimulated by the same chemical mechanism which stimulates the flow of pancreatic juice.

The intestinal juice contains at least five enzymes: enterokinase, by the action of which trypsinogen is converted into trypsin; erepsin, which produces further cleavage of the proteoses and peptones into amino acids; and the three enzymes, sucrase (invertase), maltase, and lactase, which hydrolyze respectively the three disaccharides, sucrose, maltose, and lactose.

The acidity of the chyme is, of course, neutralized by the alkalinity of the pancreatic juice, the intestinal juice, and the bile. Without attempting any technical discussion of the matter it may be said that in general under normal conditions the range of hydrogen ion activity found in the intestinal contents is favorable to the activity of the pancreatic and intestinal enzymes. Under such conditions all three classes of foodstuffs are readily acted upon by the digestive enzymes present, and brought into condition for absorption: the carbohydrates as monosaccharides; the fats as fatty acids (perhaps also monoglycerides) and glycerol; the proteins as amino acids.

Digestion in the large intestine. In the small intestine the conditions are so favorable both for digestion and for absorption, that usually very much the greater part of the available nutrients has been absorbed before the food mass reaches the ileocaecal valve.

With the passage of material from the ileum into the caecum, the caecum and ascending colon become gradually filled. Observations show that this passive filling takes place very slowly except during and immediately after meals (Hurst). The material remains in part in the large intestine for a comparatively long time (generally 18 hours or longer). During this time there is a marked absorption of water, along with the remaining products of digestion. The residual material gradually becomes more solid and takes on the character of feces.

Bacterial Action in the Digestive Tract

The digestive tract of an infant contains no bacteria at birth, but usually some gain access during the first days of life. In the average adult it is estimated that each day's food in its passage through the digestive tract is subjected to the action of over one hundred billion bacteria, chiefly in the large intestine.

Since bacteria are regularly present in the digestive tract, it has been questioned whether they may not perform some essential function in connection with the normal processes of digestion.

If it were possible to exclude absolutely all bacteria from the digestive tract, the well-being of the body would probably not be impaired; yet under such conditions as ordinarily exist, the bacteria which usually predominate in the digestive tract of the thoroughly healthy man probably render an important service in helping to protect against noxious species. *Lactobacillus acidophilus* has come to be regarded as the species best adapted to the function of maintaining a favorable condition in the human intestine. The taking of cultures of this organism and the liberal consumption of lactose and dextrin, the carbohydrates most favorable to it, may assist in the establishment and maintenance of a good condition of intestinal hygiene. And these same types of bacteria may also act to augment the body's supply of nutritionally valuable amino acids and vitamins.

Mitchell and Hamilton (1929) say: "It is a matter of first importance in practical nutrition to determine the effect of different foods and classes of foods on the intestinal flora." They then discuss favorably the method of Bergeim which measures the relative effects

of different foods upon the intestinal condition, and conclude that milk, fruit, and vegetables, as well as lactose and dextrin, tend to result in a superior intestinal hygiene; and that the value of the milk is not due simply to its lactose, for casein gave better results than meat or egg protein or even than the vegetable proteins which were investigated.

Coefficients of Digestibility of Food

The fecal matter passed per day varies considerably in health, but on an ordinary mixed diet of reasonably digestible food materials is usually between 100 and 200 grams of moist substance containing 25 to 50 grams of solids. The feces contain any undigestible substances swallowed with the food and any undigested residues of true food material; but ordinarily they appear to be largely composed of residues of the digestive juices, together with certain substances which have been formed in metabolism and excreted by way of the intestine, and bacteria, living and dead.

Prausnitz studied the feces of several persons placed alternately on meat and on rice diets and found that, although the solids of the meat were about ten times as rich in nitrogen as the solids of the rice, the two diets yielded feces whose solids were of nearly the same composition. From this point of view the feces show not so much the extent to which the food has been absorbed as whether it is a large or a small feces-former. On the other hand, so far as the nitrogen compounds of the feces are concerned, it is probably true, as generally assumed, that they represent material either lost or expended in the work of digestion, and therefore that the nitrogen of the feces is to be deducted from that of the food in estimating the amount available for actual tissue metabolism. This, however, is by no means equally true of the mineral elements, some of which, after being metabolized in the body, are eliminated mainly by way of the intestine rather than through the kidneys.

On a liberal diet consisting entirely of non-nitrogenous food, the amount of nitrogen in the feces was 0.5 to 0.9 gram per day, which is more than is sometimes found in feces from food furnishing

enough protein to meet all the needs of the body. Thus the expenditure of nitrogenous material in the digestion of fats and carbohydrates may be larger than in the digestion of protein food.

The feces always contain ether-soluble substances as well as protein. Fasting men have eliminated 0.6 to 1.3 grams per day of total lipids, a large part of which was sterols.

When the diet is very poor in fat and other lipids, the feces may contain as much as was contained in the food. As the fat content of the food rises, the actual amounts in the feces increase, but the relative amounts decrease, so that up to a certain point the apparent percentage utilization of the fat becomes higher. The limit to the amount of fat which can be thus well digested varies with the individual and with the form in which the fat is given. Quantities up to 200 grams per day have been absorbed to within 2 to 3 per cent when given in the form of milk, cheese, or butter.

In addition to protein and fat the feces always contain various other forms of organic matter which in the routine proximate analyses usually made in connection with feeding experiments are collectively reported as "carbohydrates determined by difference."

With these facts in mind one may make use of the coefficients of digestibility without being misled by them. These coefficients show the relation between the constituents of the food consumed and the corresponding constituents of the feces. Thus if the feces from a given diet contain 5 per cent as much protein as was contained in the food, this proportion is assumed to have been lost or expended in digestion, and the coefficient of digestibility of the protein of the diet is stated to be 95 per cent. While, as just shown, this assumption is not entirely correct, yet it is approximately true of the organic nutrients that the difference between the amounts in the food and in the feces represents what is available to the tissues of the body, and thus these coefficients serve a useful purpose in the computation of the nutritive values of foods.

From the results of hundreds of digestion experiments Atwater computed the average coefficients of digestibility of the organic nutrients of the main groups of food materials, when used by man as part of a mixed diet, to be as shown in Table 7.

TABLE 7. AVERAGE COEFFICIENTS OF DIGESTIBILITY OF FOODS WHEN USED IN MIXED DIET (Atwater)

	PROTEIN *Per Cent*	FAT *Per Cent*	CARBOHYDRATE *Per Cent*
Animal foods	97	95	98
Cereals and breadstuffs	85	90	98
Dried legumes	78	90	97
Vegetables	83	90	95
Fruits	85	90	90
Total food of average mixed diet	92	95	98

In some cases these figures are higher than have been reported for similar foods by other observers, the differences being due mainly to the fact (not formerly recognized) that a food may be more perfectly utilized when fed as a part of a simple mixed diet than when fed alone. Milk is an example of such a food, and has, when consumed as part of a mixed diet, a much higher coefficient of digestibility than is often assigned to it on the basis of earlier experiments.

It will be seen that the coefficients differ less for the different types of food than might be expected from popular impressions of "digestibility" and "indigestibility." It is also noteworthy that the coefficients of digestibility are less influenced by the conditions under which the food is eaten and vary less with individuals than is generally supposed. In explanation of this it may be said that general impressions of digestibility relate mainly to ease or comfort of digestion, which often bears little relation to the extent to which the food is ultimately digested in its passage through the entire digestive tract.

Enzymes of Metabolism as Distinguished from Digestion

Just as the organic reactions of our digestive processes are largely catalyzed, so are those of our metabolic processes. Thus, more or less parallel with the digestive enzymes are the tissue respiratory enzymes. And a number of body substances which catalyze other types of reaction may also properly be called enzymes and (or)

given names ending in *ase* if one wishes. There seems (1951) to be a trend in this direction, and to explain the organic reactions of the intermediary metabolism increasingly in enzymatic terms.

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Chapter 7

THE FATE OF THE FOODSTUFFS IN METABOLISM

Hopkins well said, "Among the most fundamental of the dynamic chemical events related to life are the oxidations which yield energy to the cell." Between the digestive hydrolyses of the original organic foodstuffs and the actual oxidations, there intervene not only the transportation of the nutrients but also chemical changes collectively referred to as constituting the *intermediary metabolism.* Our knowledge of some of these intermediary chemical reactions is still tentative, notwithstanding much intricate research; yet much knowledge has been gained in recent years, especially by Cori and his coworkers. The conciseness essential to the main purpose of this book forbids any attempt to follow the intricacies of the intermediary metabolism in such manner as is done in several of the works referred to at the end of the chapter. We may, however, outline the fate of carbohydrate, of fat, and of the proteins and their amino acids sufficiently to show some important relationships among them.

CARBOHYDRATE

The carbohydrate of the food, having been brought to the form of monosaccharide in the intestine, is taken up through the intestinal wall and passes thence to the circulation. Galactose, glucose, and fructose (mentioned in the order of rapidity of absorption) are taken up more readily than if the process were one of simple diffusion, the difference being sometimes indicated by the use of the term *resorption.* Or these three sugars are sometimes said to be "selectively absorbed." This fact now finds a tentative chemical explanation in the theory that their absorption is accelerated by the combination of these sugars with phosphoric acid (sometimes called

phosphorylation) to form compounds which are more quickly absorbed than are the sugars in the free state.

During rapid absorption of glucose it may reach a concentration of as much as 0.2 per cent in the portal blood; but the blood of the general circulation remains more nearly constant, usually containing a little less than 0.1 per cent of glucose. Thus it appears that a considerable part of the carbohydrate absorbed must be stored temporarily in the liver and given up gradually to the blood in the form of glucose, keeping nearly constant the glucose content of the blood of the general circulation. The carbohydrate thus stored in the liver cells is deposited in the form of glycogen, which, after abundant carbohydrate feeding, may reach 10 per cent of the weight of the liver (or, in rare cases, an even higher figure) and may fall to nearly nothing when no carbohydrate food has been taken for some time. The muscles also can store glycogen, their glycogen contents varying from traces to about 2 per cent.

The carbohydrate stored in the liver after a meal is largely converted into glucose and passes into the blood before the next meal, while the glucose content of the blood remains nearly constant. These facts indicate that the glucose of the blood must be rather rapidly used, and from our present viewpoint the outstanding question of the carbohydrate metabolism is the fate of the glucose carried to the muscles and other tissues by the blood.

The immediate fate of the glucose is influenced by the balance between the functions of two hormones: *insulin* secreted by the "islet cells" (islands of Langerhans) of the pancreas, and *epinephrine* (adrenaline, adrenine) secreted by the adrenal glands. The effects of these, and doubtless other hormones, are more or less interrelated: we can here consider only their immediate action upon the carbohydrate metabolism. Shaffer and Ronzoni consider that insulin accelerates (*a*) conversion of glucose to glycogen and (*b*) carbohydrate oxidation in liver, muscles, and probably all tissues. And they hold that epinephrine also has two chief effects: (*a*) that it causes an increase in the rate of hydrolysis of liver glycogen to glucose, an effect which insulin tends to counteract, and (*b*) that it causes an increase in the conversion of muscle glycogen to hexose

phosphate and perhaps others of the intermediates of the process of breakdown and oxidation.

Breakdown and Oxidation of Carbohydrate

It is quite probable that the sequence of chemical changes involved in the intermediary metabolism of carbohydrate, and the oxidation of the intermediates, may be different at different times and places in the body; but the outstanding feature is the breaking of the hexose (or hexose phosphate or diphosphate) into smaller molecules mostly containing three carbon atoms each. Prominent among these are: glyceric aldehyde, lactic acid, methylglyoxal (pyruvic aldehyde), pyruvic acid, and dihydroxyacetone.

It is largely the interrelationships of these three-carbon compounds, and the relative prominence of one or another of them, on which many differences of view have been expressed by those who have worked and written in this field. Critical analysis of the evidence would as above noted require more space than is here available. References may be found at the end of the chapter. The papers (there cited, and those more recently published) by the Coris and their coworkers are especially outstanding.

Also, the student will do well to keep clearly in mind that conditions vary sufficiently within the body so that more than one theory of carbohydrate metabolism may be valid, each in its proper place. The three-carbon compounds just mentioned are largely interconvertible, both among themselves and with some of the intermediates of the metabolism of fats and proteins.

Moreover, most of them are on essentially the same energy plane, as regards oxidation, with each other and with glucose so that they are easily reconvertible into glucose with changes of conditions; in fact, to a large extent may be regarded as in dynamic equilibrium with it, and (either through it or directly) with glycogen also.

Pyruvic acid may thus be regarded as an intermediate oxidation product, or as a product along with glycerol of a Cannizzaro rearrangement of two molecules of glyceric aldehyde, formed from one molecule of hexose or hexose phosphate or diphosphate. The

conversion of both pyruvic acid and glycerol into glucose may be demonstrated in the diabetic organism; also that of those intermediates—glyceric aldehyde, dihydroxyacetone, lactic acid—which have exactly the same elementary composition as glucose.

In general, the rate of oxidation (energy metabolism) in the tissues depends more largely upon the activity of the cells than upon the supply either of oxidizable matter or of oxygen. When a sufficient supply of oxygen is provided, any further increase has little effect upon the rate of combustion, and a surplus of carbohydrate instead of being burned is stored as glycogen, or converted into fat. But, while the absorption of an abundance of carbohydrate does not greatly change the rate of oxidation in the body, it may result in the use of carbohydrate as fuel almost to the exclusion of fat for the time being, as is shown by observations upon the **respiratory quotient,** which is the quotient obtained by dividing the volume of carbon dioxide given off in respiration by the volume of oxygen consumed:

$$\frac{\text{Volume of } CO_2 \text{ produced}}{\text{Volume of } O_2 \text{ consumed}} = \text{Respiratory quotient (R.Q.)}.$$

The numerical value of this quotient will evidently depend upon the elementary composition of the materials burned. Carbohydrates will yield a quotient of 1.0, since they contain hydrogen and oxygen in proportions to form water, so that all oxygen used to burn carbohydrates goes to the making of carbon dioxide, and each molecule of O_2 so consumed will yield one molecule of CO_2, occupying (under the same conditions of temperature and pressure) the same volume as the oxygen consumed to produce it.

Fats contain much more hydrogen than can be converted into water by the oxygen present in the molecule, and therefore a part of the oxygen used to burn fat goes to form water, so that the volume of oxygen consumed is greater than the volume of carbon dioxide produced. The common fats of the body and of the food give quotients approximating 0.7.

Proteins, as is evident from their elementary composition (Chapter 4), give quotients intermediate between those of carbohydrates

and fats. But if the amount of protein used in the body be determined or estimated and allowed for, one may then deduce from the respiratory quotient the proportions of carbohydrates and fats which are being burned in the body at any given time.

Storage of Carbohydrate in the Body

It has been pointed out that, when carbohydrate is absorbed in larger quantity than is required to meet the body's immediate needs for fuel, the surplus normally accumulates as glycogen, which is stored in the liver and the muscles. The amount of carbohydrate which will be stored in the entire body after rest and liberal feeding is estimated at 300 to 400 grams. Thus the total amount of carbohydrate which can be stored as such in the body is no more than is frequently taken in one day's food. When the body has no tendency to increase its store of glycogen, the further surplus of carbohydrates tends to be converted into fat.

Production of Fat from Carbohydrate

Experimental evidence of the transformation of carbohydrate into fat has been cited in Chapter 3, where it was shown that animals which fatten readily on carbohydrate food may store more body fat than could possibly be derived from the fats and proteins eaten. Also, milch cows have yielded more fat in the milk than could be accounted for on any other view than that fat was formed from carbohydrate. And there may be more carbon stored in the body from the carbohydrate food eaten by a fattening animal than can be explained in any other way than that a part of the carbon taken into the body as carbohydrate was retained as body fat.

Further proof of the ability of the animal body to change carbohydrate into fat is obtained from the respiratory quotient. As noted above, observations made after a fast tend to show quotients approaching that of fat, while after feeding carbohydrates the quotient may rise rapidly. If the quotient reaches 1.0 it shows that the body as a whole is using carbohydrate and not fat as fuel; and a quotient greater than 1.0 may be taken as evidence that the carbohydrate is

itself supplying part of the oxygen which appears as carbon dioxide, or, in other words, that it is breaking down in such a way that a part is burned while another part goes to form in the body a substance more highly carbonaceous and having a lower respiratory quotient than the carbohydrate itself. In many cases it is certain that this substance must be mainly if not entirely fat. Respiratory quotients greater than 1.0 have been observed after liberal carbohydrate feeding in several species, including man. Each such observation furnishes evidence of a conversion of carbohydrate into fat.

The formation of fat from carbohydrate in the animal body is therefore established by four distinct lines of experimental evidence: (1) by determination of the amounts of body fat formed, (2) by determination of the milk fat produced, (3) by observation of the amount of carbon stored, (4) by observations upon the respiratory quotient.

According to the Coris, there are considerable individual differences in the capacity of the body to store carbohydrate as glycogen. There also appear to be individual differences in capacity for fattening. Yet there can be few if any exceptions to the general relationship that the amount of surplus carbohydrate fuel which the body can store as carbohydrate is far exceeded by the further amount which it can store by conversion into body fat. The normal person probably can, if he wishes, gradually accumulate "stored calories" in the form of body fat up to something like one hundred times the number of calories that he can store directly as carbohydrate.

FAT

Digestion splits fats into fatty acids and glycerol (or monoglyceride) which, however, upon absorption are recombined into neutral fat. It is believed that this recombination occurs (at least in part) during the passage of these digestion products through the intestinal wall. The fat thus absorbed is taken up chiefly if not entirely by the lymph vessels, and is poured with the lymph into the blood.

After a meal rich in fat the chylomicrons (tiny fat particles) may enter the blood abundantly; but most of this fat is rapidly distributed by the blood to the tissues. Bodansky stated that when a person at rest eats one gram of fat per kilo of body weight, the concentration of fat in the blood reaches a maximum in four hours and returns to normal in six to seven hours. In general, recent work indicates that the fat content of human blood is more constant, and hence that the fat we absorb must leave our blood more rapidly, than we formerly supposed.

The fat thus leaving the blood may be burned as fuel, or stored for use as fuel in the future. When burned, its energy is used for essentially the same functions as is that of carbohydrate. The average results of a long series of very thorough experiments by Atwater and associates indicated that when both were fed as constituents of a normal mixed diet, the potential energy of fat was about 95 per cent as efficient as that of carbohydrate for the production of muscular work; while the corresponding estimate more recently reached by Krogh and Lindhard is 89 per cent.

Oxidation of Fat in the Body: β-oxidation Theory

The glycerol from fat is presumably oxidized first to glyceric aldehyde or some related three-carbon compound whose fate is then doubtless the same as when the same substance is formed in carbohydrate metabolism.

The fatty acid presents a separate problem and it is this that one has in mind in speaking of the metabolism of fat. Thus the β-oxidation theory is now generally accepted; but this does not exclude the possibility that there may be other paths of metabolism as well. Dakin remarked that while Knoop's theory of the β-oxidation of normal fatty acids is based on such a variety of evidence that its general truth can hardly be questioned, there is of course no good reason for assuming that initial oxidation of hydrogen in the β-position is the only type of initial transformation of saturated normal fatty acids that the body is capable of effecting. And Bloor (1933) has stated explicitly: "That β-oxidation is not the only method of

oxidation of the fatty acids is evident from the work of Raper and Wayne . . . and . . . of Quick." According to the β-oxidation theory, the fatty acids are attacked by oxidation at the β-carbon atom with the probable formation first of β-hydroxy, and then of β-ketonic acids. Further oxidation at this point will then cause a separation of the α- and β-carbon atoms; thus two carbons of the original fatty acid break away, presumably to undergo complete oxidation, and there remains a fatty acid with two less carbon atoms than the original. By such a process, stearic acid would yield palmitic; palmitic would yield myristic; myristic, lauric; and so on to butyric acid. Beta-oxidation of butyric acid would yield β-hydroxybutyric acid and acetoacetic acid. Normally the acetoacetic acid should yield two molecules of acetic acid which in turn should burn to carbon dioxide and water.

It has, however, been found that in diabetes or starvation, or on a diet containing too much fat and too little carbohydrate, the oxidation of acetoacetic acid is sometimes not complete. More or less acetoacetic acid, and also acetone derived from it, may accumulate in the body and appear in the excretions. This is called a condition of *ketosis*, and is explained on the basis of the view that the normal catabolism of acetoacetic acid takes place only in the presence of a simultaneous catabolism of carbohydrate, or of substances such as are formed in the intermediary metabolism of carbohydrate and also of some amino acids. This bringing about of the normal completion of the β-oxidation process, thus preventing ketosis, is called antiketogenic action.

Because of their tendency to form acetone "bodies" (substances) under certain conditions, the fatty acids are said to have ketogenic properties. Since certain amino acids, leucine, phenylalanine, and tyrosine, have also been found to be potential sources of "acetone bodies," proteins are likewise ketogenic to the degree that these amino acids are present in the protein molecule. On the other hand some of the amino acids are, like the carbohydrates, "antiketogenic" and the glycerol from fat may doubtless be converted to glucose (or equivalent intermediary products) in the course of metabolism. Consequently both fat and protein yield antiketogenic as well a

ketogenic derivatives. The relation between the total number of potential ketogenic molecules from all sources in the diet and the total number of antiketogenic molecules is known as the ketogenic-antiketogenic ratio $\left(\frac{K}{A}\right)$. Different investigators of the subject seem to attach very different degrees of importance to this ratio.

Storage of Food Fat in the Body

That fat derived from the food may be stored as body fat has already been shown (Chapter 3) and need not be discussed further here. Mills (1911) found that fatty oils injected with antiseptic precautions into the subcutaneous tissue may, under favorable conditions, be absorbed therefrom and used in the body in the same way as if obtained by feeding; but Koehne and Mendel found further that fatty oils introduced parenterally were too slowly distributed and metabolized to be a dependable source of any considerable proportion of the fuel needed for the support of the energy requirements in nutrition.

As noted in Chapter 3, experiments with deuterium fats emphasize the extent to which the absorbed fatty acids go into the body's fat stores before undergoing oxidation.

Whether fat once deposited will remain and accumulate or be returned to the circulation and used as fuel, will depend upon the balance between the food consumption and the food requirements of the body as a whole. In this respect, there is no known difference between fat consumed and deposited as such and fat formed in the body from other food materials.

PROTEIN

It is now believed that the hydrolysis of proteins to amino acids in the digestive tract is, in normal cases, practically complete. The significance of this digestive cleavage lies not simply in the formation of more soluble and more readily diffusible substances, but also in the resolution (transformation) of the complex molecules of food

protein into their simple amino-acid "building stones," which may be very extensively rearranged by the body in the synthesis of its own tissue proteins; or in such specialized proteins as those of milk.

Absorption and Distribution of Protein Digestion Products

The amino acids resulting from digestive hydrolysis of the food proteins pass through the intestinal wall and into the blood and are thus distributed throughout the body. They are rapidly absorbed from the blood into the various tissues. Thus each tissue receives its protein material in the form of amino acids from which can be synthesized the particular kind of protein characteristic of the tissue in question. In other words each tissue makes its own proteins from the amino acids brought by the blood.

Utilization of Protein in the Tissues

The proteins of the digested food, absorbed and distributed in the form of amino acids as described above, soon become available for nutrition; and among other functions they, like the carbohydrates and fats, may be oxidized as fuel for muscular work. (It will of course be understood that the protein is not supposed to be oxidized directly. Protein is split into amino acids, the amino acids de-aminized, and the non-nitrogenous residues of the amino acids are oxidized.) Pflüger showed that protein may serve as a source of muscular energy by feeding a dog for 7 months exclusively upon meat practically free from fat and carbohydrate, and requiring it throughout the experiment to do considerable amounts of work, the energy for which must in this particular case have been derived largely from the protein consumed.

The experimental facts and theoretical explanations regarding the intermediary metabolism of proteins (or of the amino acids arising from them) in the body tissues must now be considered. By experiment it has been found that if a meal extra rich in protein be eaten, an increased elimination of nitrogenous end products can be observed within 2 or 3 hours, and probably much the greater

part of the surplus nitrogen will have been excreted within 24 hours of the time it was taken into the stomach. It does not follow, however, that the surplus nitrogen ingested supplies (directly and literally)* all of the extra nitrogen excreted or that the whole of the protein molecule is broken down and eliminated at the same rate. Evidently, the nitrogenous radicles of the protein may be split off in such a way as to leave a non-nitrogenous residue in the body, and the study of protein metabolism involves a consideration of the fate of both the nitrogenous and non-nitrogenous derivatives.

Chemistry of the Deaminization

It is probable that the deaminization may occur, according to the conditions existing at the place and time, in any of three ways. Thus the typical amino acid, alanine, may undergo simple, oxidative, or hydrolytic deaminization to yield methylglyoxal, pyruvic acid, and lactic acid, respectively. From what we have already seen of the intermediary metabolism of carbohydrate, we know that these non-nitrogenous derivatives of protein may in part be changed into carbohydrate in the body. This fact is confirmed by much direct experimental evidence.

Formation of Carbohydrate from Protein

As early as 1876 Wolffberg tested the formation of carbohydrate from protein by fasting fowls for two days in order to free them from glycogen and then feeding for two days with meat powder which had been washed free from carbohydrate. Two of the fowls were killed soon after this protein feeding and showed more glycogen in their livers and muscles than could be accounted for except as derived from the protein fed. This formation of glycogen from protein was fully confirmed by Kulz in a long series of experiments in which the food consisted of chopped meat thoroughly extracted

* In the sense sometimes called "bookkeeping with the body" the extra excretion is entirely *attributable to* the extra intake; but as previously inferred from the concept of dynamic equilibria, and now directly demonstrated in experiments with isotopic nitrogen, there is always some *exchange of material.*

with warm water (Lusk). Much additional evidence of the production of carbohydrate from protein could be cited.

The most striking evidence of the formation of carbohydrate from protein in the animal body is found in the many observations and experiments which have been made in cases of diabetes, and in experimental glycosuria produced either by administration of phlorizin or by removal of the pancreas. In such cases large amounts of carbohydrate may be excreted in the form of glucose even when there is little body fat, and no carbohydrate is fed. Much of the glucose must therefore result, in these experiments, from the metabolism of protein. In Lusk's exhaustive experiments upon dogs rendered diabetic by phlorizin, about 58 per cent of the total weight of protein broken down in the body (whether in fasting or on a meat diet) was eliminated in the form of glucose.

Production of Fat from Protein

At various times there has been much controversy regarding the formation of fat from protein in the animal body. As there has long been abundant experimental evidence of the production of carbohydrate from protein and of the transformation of carbohydrate into fat, it was and is evident that protein food can in one way or another contribute to the formation of fat in the body. "The α-hydroxy or α-ketonic acids resulting from the deamination of amino acids may evidently be oxidized directly, or they may be converted into sugar and stored as glycogen, or converted into fats" (Mitchell and Hamilton). There is, however, always an added satisfaction when the experimental evidence is made more direct, and this has now been done by Longenecker (1939) and by Hoagland and Snider (1939).

The Fate of the Nitrogen in Protein Metabolism

Urea and ammonia. While the work of Van Slyke and others indicates a more complex chemistry than formerly supposed, urea and ammonia are interconnected as end-products of the protein metabolism, and together usually account for about nine tenths of

the urinary nitrogen. In health and on a liberal protein diet, from 82 to 88 per cent of the total nitrogen excreted by the kidneys is usually in the form of urea. On a low protein diet this percentage is lower. Normally, about 2 to 6 per cent of the total nitrogen of the urine is in the form of ammonium salts, the amount depending largely upon the relation between the amounts of acid-forming and of base-forming elements in the food, which will be discussed in Chapter 13.

Uric acid and the purine bases (nucleic acid metabolism). A part of the nitrogen of human urine is always in the form of uric acid and purine bases. These owe their origin either to the free purine substances of the food, such as the guanine and hypoxanthine of meat extract, or to the metabolism of nucleic acid derived from the nucleoproteins of the food or of the body tissues. The constituent groups of the nucleic acids, their modes of linkage, and the order of their liberation on hydrolytic cleavage such as occurs in metabolism have been very fully discussed by Levene and Bass (1931).

Purines undergoing metabolism in the body may be derived either (1) from the nucleoprotein of body tissue or (2) from the food, which may contain both nucleoproteins and free purines. On ordinary mixed diet the total urinary output of uric acid averages about 0.6 to 0.7 gram per man per day, in which case the uric acid nitrogen constitutes about 1 to 3 per cent of the total nitrogen of the urine.

Creatine and creatinine. Chemically, creatinine is the anhydride of creatine. The biochemical relationships and nutritional significance of these substances have been much discussed and the literature is far too extensive to be summarized satisfactorily here, except in the aspect with which we are here directly concerned. See Rose (1933, 1935), and Bodansky (1938).

The quantity of creatinine excreted is fairly constant for the individual, averaging about 0.02 gram per kilogram of body weight per day. On ordinary mixed diet the creatinine nitrogen usually constitutes 3 to 7 per cent of the total nitrogen of the urine.

Distribution of excreted nitrogen as influenced by level of protein metabolism. The above statements regarding the distribution of the

eliminated nitrogen among the different end products refer to results obtained upon an ordinary mixed diet containing an average amount of protein. Folin showed, by a careful and extended study of the urines of healthy men living first upon high and then upon low protein diets, that the distribution of the nitrogen between urea and the other nitrogenous end products depends very largely upon the absolute amount of nitrogen metabolized (level of protein metabolism). In the case of a man who on one day consumed high protein diet free from meat, and a week later was living on a diet of starch and cream which furnished in all about 6 grams of protein per day, the distribution of end products was changed as shown in Table 8.

TABLE 8. NITROGEN DISTRIBUTION IN URINE AS INFLUENCED BY LEVEL OF PROTEIN INTAKE (Folin)

	ON HIGH PROTEIN DIET (Free from Meat)		ON LOW PROTEIN DIET (Starch and Cream)	
	Grams	*Per Cent*	*Grams*	*Per Cent*
Total nitrogen	16.8	—	3.6	—
Urea nitrogen	14.7	87.5	2.2	61.7
Ammonia nitrogen	0.49	2.9	0.42	11.3
Uric acid nitrogen	0.18	1.1	0.09	2.5
Creatinine nitrogen	0.58	3.6	0.60	17.2
Undetermined nitrogen	0.85	4.9	0.27	7.3

Thus, on passing from the high protein to the low protein diet (both being free from meat products) there was a marked decrease in both the absolute and the relative amounts of urea; and a decrease in the absolute, but increase in the relative, amount of uric acid; while the absolute amount of creatinine remained practically unchanged so that its relative amount was greatly increased.

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Chapter 8

THE FUEL VALUE OF FOOD AND THE ENERGY REQUIREMENT OF THE BODY

We have seen that, under normal conditions, carbohydrate after its absorption into the body may either be oxidized, or stored as glycogen, or transformed into fat; that fat may be oxidized or stored and that at least its glyceryl radicle may be converted into carbohydrate; and that protein absorbed as amino acids may either be built up into body protein, or deaminized and oxidized, or may yield carbohydrate, or may contribute to the production of fat. It has also been shown that any or all of these foodstuffs may be utilized as fuel for muscular work.

Thus the body is not restricted to the use of any one foodstuff for the support of any one kind of work, but on the contrary has very great power to convert one nutrient into, or use it in place of, another, and so to utilize its resources that the total potential energy of all of these nutrients is economically employed to support the work of all parts of the organism. The carbohydrates, fats, and proteins stand in such close mutual relations in their service to the body that for purposes of providing energy we may properly consider the food as a whole with reference to the total nutritive requirements. Since the most conspicuous nutritive requirement is that of energy for the work of the body, and since these organic nutrients all serve as fuel to yield this energy, one basis of comparison is that of energy value.

The energy value of food is usually and conveniently expressed in terms of heat units (Calories); but it is important to realize that the body is not a heat engine. In a heat engine, heat is the source of the work; in the body the heat is rather the result of the internal and external work which the body does. With this clearly in mind there need be no real misconception in using heat units to express

the energy values of foods and the energy requirements of the body.

Heats of Combustion of the Foodstuffs

The calorific value or heat of combustion of any substance, i.e., the amount of energy liberated (transformed) by the burning of a given quantity of the combustible material, is best determined by means of the bomb calorimeter, the original form of which was devised by Berthelot. The particular form of Berthelot bomb which was most used in the quantitative development of the fundamental energy relations of food materials and physiological products was that of Atwater and Blakeslee, fully described by Atwater and Snell in the *Journal of the American Chemical Society* for July, 1903. In outline (Fig. 5) it consists of a heavy steel bomb, *A*, with a platinum or gold-plated copper lining and a cover held tightly in place by means of a strong screw collar. A weighed amount of sample, usually pressed into pellet form, is placed in a capsule, *B*, within the bomb, which is then closed except for the oxygen valve, charged with oxygen to a pressure of at least 20 atmospheres (300 pounds or more to the square inch). The oxygen valve is then closed, and the

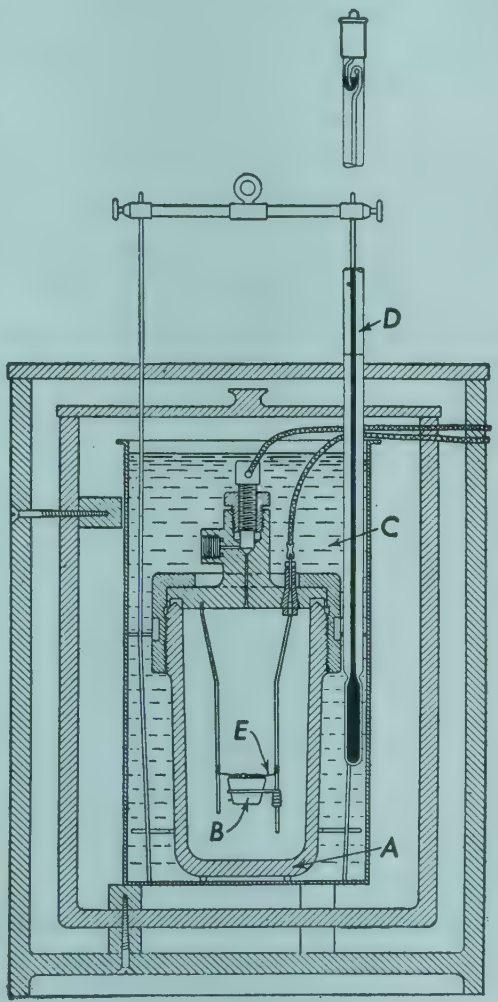

FIG. 5. The Atwater bomb calorimeter.

bomb immersed in a weighed amount of water, *C*. The water is constantly stirred and its temperature taken at intervals of one minute by means of a differential thermometer, *D*, capable of being read to one thousandth of a degree. After the rate at which the temperature of the water rises or falls has been determined, the sample is ignited by means of an electric fuse, *E*, and, on account of the large amount of oxygen present, undergoes rapid and complete combustion. The heat liberated is communicated to the water in which the bomb is immersed, and the resulting rise in temperature is accurately determined. The thermometer readings are also continued through an "after period," in order that the "radiation correction" may be calculated and the observed rise of temperature corrected accordingly. This corrected rise, multiplied by the total heat capacity of the apparatus and the water in which it is immersed, shows the total heat liberated in the bomb. From this must be deducted the heat arising from accessory combustions (the oxidation of the iron wire used as a fuse, etc.) to obtain the number of Calories * arising from the combustion of the sample.

The necessity for corrections for heat loss is avoided in the adiabatic form of the bomb calorimeter which is now generally used.

Another apparatus for determination of the energy value of foods is the oxy-calorimeter, devised by Benedict and coworkers, which measures the volume of oxygen required to burn a known weight of the food. From this, by means of factors established by use of the bomb calorimeter, the calorific value of the food is calculated. The factors for converting liters of oxygen to calories as given by Benedict and Fox in *Industrial and Engineering Chemistry*, Vol. 17, page 912 (1925), may also be found in Appendix A of previous editions of this book.

The heat of combustion of organic substances is closely connected with their elementary composition. One gram of carbon burned to carbon dioxide yields 8.08 Calories and 1 gram of hydrogen burned

* When the term Calorie is used in this book it will be understood to mean the large calorie, or kilogram calorie, i.e., the amount of heat required to raise the temperature of one kilogram of water one degree Centigrade. This is very nearly the same as the heat required to raise four pounds of water one degree Fahrenheit.

to water yields 34.5 Calories. If a compound consisting of carbon and hydrogen only be burned, it gives nearly the amount of heat which these would give if burned separately.

On the other hand, in carbohydrates and fats, which are composed of carbon, hydrogen, and oxygen, the carbon and hydrogen are already partly oxidized by the oxygen present in the molecule; so that 100 grams of glucose, for example, containing 40 grams carbon, 6.7 grams hydrogen, and 53.3 grams oxygen, would yield considerably less heat than would be obtained by burning 40 grams of pure carbon and 6.7 grams of pure hydrogen to carbon dioxide and water respectively.

Proteins when burned in the calorimeter give off their carbon as carbon dioxide, their hydrogen as water, and their nitrogen as nitrogen gas.* Thus the nitrogen contributes nothing to, and takes nothing from, the heat of combustion; and the latter is essentially dependent here, as in the case of carbohydrates and fats, upon the amounts of carbon and hydrogen present and the extent to which

TABLE 9. HEATS OF COMBUSTION AND ELEMENTARY COMPOSITION OF TYPICAL COMPOUNDS

SUBSTANCE	HEAT OF COMBUSTION	CARBON	HYDROGEN	OXYGEN	NITROGEN	SULFUR	PHOSPHORUS
	Calories per Gram	*Per Cent*	*Per Cent*	*Per Cent*	*Per Cent*	*Per Cent*	*Per Cent*
Glucose	3.75	40.0	6.7	53.3	—	—	—
Sucrose	3.96	42.1	6.4	51.5	—	—	—
Starch } Glycogen }	4.22	44.4	6.2	49.4	—	—	—
Body fat	9.60	76.5	12.0	11.5	—	—	—
Butter fat	9.30	75.0	11.7	13.3	—	—	—
Edestin	5.64	51.4	7.0	22.1	18.6	0.9	—
Legumin	5.62	51.7	7.0	22.9	18.0	0.4	—
Gliadin	5.74	52.7	6.9	21.7	17.7	1.0	—
Casein	5.85	53.1	7.0	22.5	15.8	0.8	0.8
Albumin	5.80	52.5	7.0	23.0	16.0	1.5	—
Gelatin	5.30	50.0	6.6	24.8	18.0	0.6	—
Creatinine	4.58	42.5	6.2	14.1	37.2	—	—
Urea	2.53	20.0	6.7	26.7	46.6	—	—

* As a matter of fact a small part of the nitrogen is oxidized to nitric acid in the bomb calorimeter, but this is determined and its heat of formation subtracted, so that the final results are as stated above.

they are already combined with oxygen. A little additional heat is obtained by the burning of the small amount of sulfur present in the protein.

The relation between the elementary composition and heat of combustion will be made clearer by the study of Table 9, which includes a number of typical compounds found in the food or formed in the body.

Since the energy used in the body is obtained from the oxidation of the same kinds of compounds which exist in food, i.e., from carbohydrates, fats, and proteins (or their cleavage products), we can estimate the amount of energy transformed in the body if we know the amount of each kind of foodstuff oxidized. Account must, however, be taken of the completeness of the oxidation in each case.

When undergoing complete oxidation in the bomb calorimeter, the foodstuffs yield the following average heats of combustion:

Carbohydrates	4.1 Calories per gram [a]
Fats	9.45 Calories per gram [a]
Proteins	5.65 Calories per gram [a]

[a] These are weighted averages, taking account of relative amounts of different carbohydrates, different fats, and different proteins in ordinary food supplies.

In the body, carbohydrates and fats are oxidized to the same products as in the calorimeter and so yield the same amounts of heat. Protein, however, which burns in the calorimeter to carbon dioxide, water, and nitrogen, yields in the body no free nitrogen, but urea and other organic nitrogen compounds which are eliminated as end products. These organic nitrogenous end products are combustible; they represent a less complete oxidation of protein in the body than in the bomb calorimeter. The loss of potential energy calculated on the assumption that all nitrogen left the body as urea would be about 0.9 Calorie per gram of protein, but on account of the elimination of other substances of higher heat of combustion (creatinine, uric acid, etc.), the actual loss in the form of combustible end products is considerably greater and averages about 1.3 Calories for each gram of protein metabolized in the body.

Hence, when the body burns material which it has previously absorbed, it obtains:

From carbohydrates	4.1 Calories per gram
From fats	9.45 Calories per gram
From protein (5.65 − 1.30 =)	4.35 Calories per gram

In calculating the fuel value of the food, however, allowance must be made for the fact that a part of each of the materials is lost in digestion, as explained in Chapter 6.

The approximate averages on a mixed diet are:

Carbohydrates	2% lost, 98% absorbed
Fats	5% lost, 95% absorbed
Protein	8% lost, 92% absorbed

The approximate *physiological fuel values* of the food constituents are then:

Carbohydrates	4.1 × 98% = 4. Calories per gram
Fats	9.45 × 95% = 9. Calories per gram
Protein	4.35 × 92% = 4. Calories per gram

These physiological fuel values are known as the Atwater or Atwater and Bryant factors for calculating fuel values of food.

During and since World War II a need has been felt for *more specific physiological energy factors* for individual items or smaller food-groups than those averaged by Atwater.

The Bureau of Human Nutrition and Home Economics, using Atwater's data in conjunction with those of the Food and Agriculture Organization and from other sources, compiled the more specific data here shown in Table 10, reproduced by permission from Taylor and MacLeod (1949, pages 206–208).

In general the energy values per unit weight of food as computed by these more specific factors are slightly higher for animal foods and refined vegetable foods and lower for the coarser vegetable foods including roots and whole grain products, than the values yielded by the Atwater factors.

The small difference between them should not be disconcerting, and for many purposes this difference may be ignored.

TABLE 10. PHYSIOLOGICAL ENERGY FACTORS MORE SPECIFIC THAN THOSE OF ATWATER

FOOD OR FOOD GROUP	PROTEIN	FAT	CARBOHYDRATE (by difference)
	Cal. per Gm.	*Cal. per Gm.*	*Cal. per Gm.*
Milk, milk products	4.27	8.79	3.87
Meat, fish	4.27	9.02	—
Eggs	4.36	9.02	—
Separated fats			
Butter fat	—	8.79	—
Other animal fats	—	9.02	—
Vegetable fats and oils, hydrogenated fats, margarine	—	8.84	—
Cereals			
Barley, light	3.55	8.37	3.95
Cornmeal, whole ground	2.73	8.37	4.03
Cornmeal, degerminated	3.46	8.37	4.16
Crackers, soda, plain	4.23	8.37	4.12
Macaroni	3.91	8.37	4.12
Noodles (with egg)	3.91	8.84	4.12
Oatmeal (rolled oats)	3.69	8.37	4.12
Rice, polished	3.78	8.37	4.12
Rye flour, whole grain	2.41	8.37	3.78
Wheat, 97–100% extraction	3.59	8.37	3.78
Wheat, 85–93% extraction	3.78	8.37	3.95
Wheat, 70–74% extraction	4.05	8.37	4.12
Other cereals, refined	3.87	8.37	4.12
Other foods			
Dry beans, peas, nuts	3.47	8.37	4.07
Potatoes and starchy roots	2.74	8.37	4.03
Other underground crops [a]	2.74	8.37	3.84
Other vegetables	2.44	8.37	3.57
Fruits	3.36	8.37	3.60
Lemons, lemon juice	3.36	8.37	2.70
Sugar (disaccharide)	—	—	3.87
(glucose)	—	—	3.68

[a] Vegetables such as beets, carrots, onions, parsnips, radishes.

Fuel Value of Food in Nutrition

If the composition of a food is known, its approximate fuel value is easily computed using the Atwater factors. Thus milk of about average composition contains: *

* In this book, statements of proximate composition of foods are based on the Federal revised averages as given in the United States Department of Agriculture Handbook No. 8 (1950–51). Figures for the energy values of

Protein, 3.5 per cent; fat, 3.9 per cent; carbohydrate, 4.9 per cent.

One hundred grams of such milk will furnish in the form of protein, (3.5 × 4. =) 14.0 Calories; of fat, (3.9 × 9. =) 35.1 Calories; of carbohydrate, (4.9 × 4. =) 19.6 Calories; total for 100 grams of milk, 68.7 Calories. Or, if greater precision were desired, one might multiply the percentage of protein in milk by its "more specific physiological energy factor" (from Table 10), 4.27; the percentage of fat by 8.79; and that of carbohydrate by 3.87.

Eggs are estimated to contain, on the average, in the edible portion, 12.8 per cent of protein, 11.5 per cent of fat, and 0.7 per cent of carbohydrate. They would then furnish, per 100 grams, approximately 158 Calories.

As sources of energy, the quantities of foods to be taken as equivalent or mutually replaceable are those which furnish equal fuel value, e.g., 100-Calorie portions, the weights of which may be calculated directly from the fuel values of 100 grams.

Thus, for milk: 100 grams furnish 68.7 Calories; then if x be the number of grams which furnish 100 Calories:

$$100 : 68.7 :: x : 100;\ x = 146.$$

Similarly for eggs:

$$100 : 158 :: x : 100;\ x = 63.$$

And since the two extremes in the proportion are always the same, the weight in grams of the 100-Calorie portion may always be found by dividing 10,000 (the product of the extremes) by the number of Calories per 100 grams.

The fuel value of foods is often stated in Calories per pound. Thus the fuel value of milk is given as 310 to 325 Calories per pound.*

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foods in Table 65 of the appendix are taken from this same government publication, and were computed by the use of the "more specific factors." In portions of the present text, however, the more traditional Atwater factors are still employed, as they were in the work which it describes.

* For most purposes the difference between 310 and 325 Calories per pound may be ignored and Atwater or more specific factors used interchangeably. One need not be disconcerted thereby.

Since 453.6 grams furnish 310 Calories,

$$453.6 : 310 :: x : 100;\ x = 146,$$

— the number of grams required to furnish 100 Calories. Hundred-Calorie portions of several typical foods, with the distribution of the Calories between protein, fat, and carbohydrate, are shown in Table 11. *Hundred Calorie Portions* of a large number of foods may be found in Taylor and MacLeod's revision of Rose's *Laboratory Handbook for Dietetics* (Macmillan).

TABLE 11. HUNDRED-CALORIE PORTIONS OF TYPICAL FOODS. (Atwater)

FOOD (Edible Portion)	WEIGHT OF 100-CALORIE PORTION		DISTRIBUTION OF CALORIES		
	Grams	*Ounces*	*In Protein*	*In Fat*	*In Carbohydrate*
Almonds	16	0.6	11.6	76.1	12.3
Apples	156	5.5	1.9	5.6	92.5
Beans, dried	29	1.0	25.2	3.9	70.9
Beans, snap or string	237	8.4	22.7	4.3	73.0
Butter	14	0.5	0.3	99.5	0.2 [a]
Cabbage	350	12.4	19.6	6.3	74.1
Carrots	224	7.9	10.7	6.0	83.3
Eggs	64	2.3	32.5	65.7	1.8
Grapefruit	226	8.0	4.5	4.1	91.4
Kale	201	7.1	31.3	10.8	57.7
Lettuce	549	19.4	26.4	9.9	63.7
Milk	146	5.2	20.4	51.1	28.5
Oils, salad	11	0.4	—	100.	—
Oranges	199	7.0	7.2	3.6	89.2 [b]
Peas	99	3.5	26.5	3.6	69.9
Potatoes	117	4.1	9.4	1.0	89.6
Sugar, granulated	25	0.9	—	—	100.
Tomatoes	441	15.6	17.6	11.9	70.5 [b]

[a] Includes both the lactose and the lactic acid of that part of the buttermilk which the butter retains.

[b] As is usual in calculations of this kind, organic acids are here counted with carbohydrates.

Since proteins and carbohydrates have nearly the same average fuel value and the ash of food does not as a rule constitute a large

percentage, the striking differences in the energy values of foods per 100 grams and in the weights of the various foods required to furnish 100 Calories are chiefly referable to differences in water content or fat content or both. That dry beans have nearly 8 times the fuel value of snap beans or carrots is essentially due to the difference in moisture, while the difference in fuel value between lean beef and bacon, or between codfish and salmon, is chiefly a matter of fat content.

Meat free from fat is about three fourths water and one fourth protein, and so has a fuel value of about one Calorie per gram, while clear fat has a fuel value about nine times as great.

Fuel values of meats as given in the standard tables are apt to be somewhat misleading, inasmuch as they allow for all the fat ordinarily found on the various cuts as taken from the animal, whereas in many cases a considerable part of this fat is trimmed off by the butcher and treated as a by-product; and often much of the remaining fat is removed either in the kitchen or at the table. If a pound of steak consists of 14 ounces of clear lean and 2 ounces of clear fat, and the fat is not eaten, at least half of the total fuel value of the pound of steak is lost.

Many vegetables are more watery than lean meats and so contrast even more strikingly with the fats. An ounce of clear fat pork is equal in fuel value to about two pounds of cabbage or of kale; an ounce of olive oil to over three pounds of lettuce. On the other hand, however, the lettuce and the cabbage, and in still higher degree the kale, have important mineral and vitamin values of which olive oil and clear fat pork have little if any.

Energy Requirement—Methods of Study and Amounts Required for Maintenance at Rest

We have every reason to suppose that under ordinary conditions the carbohydrates, fats, and proteins each supply the body with the kinds of energy needed for its maintenance and for its work, approximately in proportion to their fuel values as calculated above. We do not now believe that any one nutrient is used to the exclusion of

others as a source of energy for any particular function, nor indeed that the body makes any great distinction among the foodstuffs as sources of energy. The fuel value of the diet as a whole is utilized to meet the energy requirements of the whole body. For the present, therefore, it is the fuel value of the day's dietary which we have to consider rather than the distribution of this as regards protein, fat, and carbohydrate.

The total food (or energy) requirement is best expressed in Calories per day, either for the whole body or per kilogram of body weight, and for convenience of discussion it is usually assumed that the average body weight (without clothing) is for men 70 kilograms (154 pounds) and for women eight tenths as much, 56 kilograms (123 pounds).

There are four important methods of studying the food requirements of man: *

(1) By observing the amount of food consumed (dietary studies).

(2) By observing the amount of oxygen consumed, or carbon dioxide produced, or both (respiration experiments).

(3) By determining the balance of intake and output (carbon and nitrogen balance experiments).

(4) By direct measurement of heat given off by the body (calorimeter experiments).

1. Dietary studies. Probably most dietary studies give little more than a general indication of the food habits of the people studied; but in cases where persons have maintained for a long time the same dietary habits and other conditions of life, and the body weight has remained practically constant, it may be fairly safe to assume that the food has furnished just about the right amount of energy for the maintenance of the body under the observed conditions.

Great care must be taken in drawing inferences from the body weight because of the readinesss with which the body gains or loses

* For an account of the historical development of the principles which underlie the measurement of metabolism see the introductory chapter of Lusk's *Elements of the Science of Nutrition.*

moisture. Athletes often lose 2 or 3 pounds in an hour of vigorous exercise, and regain it, through food and drink, in less than a day. Gain or loss of body weight during short periods, therefore, does not by any means necessarily imply a corresponding gain or loss of fat. When, however, the weight remains nearly the same for months at a time, it may usually be assumed that there is no important gain or loss of tissue and that the body is receiving just about the proper total amount of food for its needs. Under these conditions an accurate observation of the food consumed may give valuable indications as to the actual food requirement. Of such dietary studies perhaps the most useful individual example is that of Neumann, who reduced his diet to what appeared to be just about sufficient for his needs and then recorded all food and drink taken during a period of 10 months in which the body weight remained nearly constant. The average daily food furnished: *

TABLE 12. DATA OF NEUMANN'S FIRST DIETARY STUDY

Nutrients	*Weight*	*Factors*		*Calories*	*Total Calories per Day*
Protein	66.1 grams	× 4.	=	264.4	2242
Fat	83.5 grams	× 9.	=	751.5	
Carbohydrate [a]	306.5 grams	× 4.	=	1226.0	

[a] Including some alcohol (taken in the form of beer), which is estimated as equivalent in fuel value to 1.75 times its weight of carbohydrate.

The 2242 Calories per day were evidently fully sufficient to meet the energy requirements of this man, whose weight was 66.5 to 67 kilograms (about 147 pounds) and who was engaged at his usual (mainly sedentary) professional work in the Hygienic Institute at Kiel.

Later, when his weight had increased to 71.5 kilograms (157 pounds) as the result of following for a time a more liberal diet (furnishing about 2600 Calories per day), he again observed his dietary while taking what was supposed to be an amount of food sufficient for the maintenance of the body and no more. This second dietary study was continued for 8 months, during which the average daily food consumption was found to be:

* The data are from Chittenden's *Nutrition of Man*, page 286.

TABLE 13. DATA OF NEUMANN'S SECOND DIETARY STUDY

Nutrients	*Weight*	*Factors*		*Calories*	*Total Calories per Day*
Protein	76.2 grams	× 4.	=	304.8	2000
Fat	109.0 grams	× 9.	=	981.0	
Carbohydrate [a]	178.6 grams	× 4.	=	714.4	

[a] Including some alcohol (taken in the form of beer), which is estimated as equivalent in fuel value to 1.75 times its weight of carbohydrate.

The body weight remained nearly constant.

These results indicate that this subject, a man of average size, living a normal professional life involving no manual labor in the ordinary sense, but not excluding such muscular movements as are naturally incidental to a sedentary occupation, found his energy requirements satisfied with food furnishing 2000 to 2250 Calories per day.

2. Respiration experiments. Since the foodstuffs yield their energy through being oxidized in the body, it is evident that a measure of the energy metabolism can be obtained by finding either the amount of foodstuffs oxidized or the amount of oxygen which is consumed in the process. The apparatus devised and used by Zuntz for this purpose provides a mask, fitting air tight over the mouth and nose and connected by means of valved pipes with apparatus for measuring and analyzing the inspired and expired air. In this way one can determine the volume of oxygen entering, and the volume leaving, the lungs. The difference is the volume consumed in the body.

Benedict devised an improved form of respiration apparatus in which the subject breathes, either through a mouth- or nose-piece, from a current of air which is purified and kept in circulation in the same manner as that of the respiration calorimeter chamber described below. The carbon dioxide which the man produces is absorbed quantitatively and the oxygen which he consumes is measured by the change in level of a spirometer previously filled with oxygen gas which drops as oxygen is consumed by the man.

A given volume of oxygen used in the body may liberate somewhat different amounts of heat, according as it oxidizes fat, carbohydrate

or protein. For accurate estimations of the energy liberated it is therefore necessary to know the kind of material oxidized, as well as the amount of oxygen consumed. This is calculated from the respiratory quotient as explained in Chapter 7.

Since the amount of protein broken down in the body can be estimated from the nitrogen excretion, the determination of the respiratory quotient along with the oxygen consumption shows the extent of the combustion in the body and the proportions of fat and carbohydrate burned. From these data the energy can be calculated.

As a matter of fact it is not necessary to go through the actual calculation of the amounts of fat and carbohydrate burned, since the energy derived from a liter of oxygen when used to burn carbohydrate and fat in different proportions can be calculated once for all and expressed in relation to the respiratory quotient as shown in Table 14.

It is then only necessary to determine the respiratory quotient, or if working under conditions for which it is known, the volume of oxygen consumed, in order to know the number of Calories of energy metabolized. This is sometimes called the method of *indirect calorimetry*. For descriptions of other forms of respiration apparatus see DuBois' *Basal Metabolism in Health and Disease.*

This method of studying the energy metabolism permits of experiments being carried out very quickly, and is therefore especially useful for the direct investigation of conditions which affect energy metabolism promptly, such as muscular work or the eating of food. The periods of observation cannot be very long, but the probable results for the 24 hours' metabolism can be estimated by the data obtained during frequent short periods at different times of the day and night.

From the results of many observations by the Zuntz method, Magnus-Levy estimated the minimum metabolism of a man of average size kept absolutely motionless and fasting at 1625 Calories per day. Food barely sufficient for maintenance would increase this by 175 Calories, and such incidental muscular movements as would ordinarily be made by a man at rest in bed would involve another

TABLE 14. ENERGY VALUES OF OXYGEN AND CARBON DIOXIDE AT DIFFERENT RESPIRATORY QUOTIENTS (Zuntz and Schumburg)

RESPIRATORY QUOTIENT	CALORIES PER LITER OF OXYGEN	CALORIES PER LITER OF CARBON DIOXIDE	CALORIES PER GRAM OF CARBON DIOXIDE
0.70	4.686	6.694	3.408
0.71	4.690	6.606	3.363
0.72	4.702	6.531	3.325
0.73	4.714	6.458	3.288
0.74	4.727	6.388	3.252
0.75	4.739	6.319	3.217
0.76	4.752	6.253	3.183
0.77	4.764	6.187	3.150
0.78	4.776	6.123	3.117
0.79	4.789	6.062	3.086
0.80	4.801	6.001	3.055
0.81	4.813	5.942	3.025
0.82	4.825	5.884	2.996
0.83	4.838	5.829	2.967
0.84	4.850	5.774	2.939
0.85	4.863	5.721	2.912
0.86	4.875	5.669	2.886
0.87	4.887	5.617	2.860
0.88	4.900	5.568	2.835
0.89	4.912	5.519	2.810
0.90	4.924	5.471	2.785
0.91	4.936	5.424	2.761
0.92	4.948	5.378	2.738
0.93	4.960	5.333	2.715
0.94	4.973	5.290	2.693
0.95	4.985	5.247	2.671
0.96	4.997	5.205	2.650
0.97	5.010	5.165	2.629
0.98	5.022	5.124	2.609
0.99	5.034	5.085	2.589
1.00	5.047	5.047	2.569

200, making a total of 2000 Calories as the estimated food requirement of a man at rest with a maintenance diet. Magnus-Levy further estimated that the man, if doing no work (in the ordinary sense), but allowed to move about the room instead of remaining in bed, would require 2230 Calories per day.

3. Carbon and nitrogen balance experiments. From a comparison of the constituents of the food consumed ("intake") and of the substances eliminated from the body ("output"), the material actually oxidized and the energy liberated in the oxidation may be determined.

The intake is found by weighing and analyzing all food eaten; the output by collecting and determining the end products eliminated through the lungs, the kidneys, the intestines, and sometimes the skin. The time unit in experiments upon the intake and output is almost always 24 hours, the experimental day beginning preferably just before breakfast. The feces belonging to the experimental days are marked, usually by giving a small amount of lampblack with the food as in ordinary digestion experiments, separated, and analyzed. The end products given off by the lungs and kidneys during an experimental day are taken as measuring the material broken down in the body during the same period.

Some time is of course required for the elimination of the nitrogenous end products through the kidneys. The unavoidable "lag" in the elimination of nitrogen may introduce an error in interpreting the nitrogen balance unless the subject has been kept for a few days in advance upon the same diet which is to be used in the experiment.

Assuming that the total nitrogen and carbon of the absorbed food existed in the form of protein, fat, and carbohydrate, and that the amount of carbohydrate in the body is the same at the beginning and end of each experiment, it is only necessary to determine the carbon dioxide of the expired air and the carbon and nitrogen of the waste products, in order to calculate the amounts of material oxidized and of energy liberated in the body. Experiments of this sort have played an important part in the development of our knowledge of nutrition. The calculations are usually based on the average analyses of protein and body fat shown in Table 15.

TABLE 15. AVERAGE ELEMENTARY COMPOSITION OF PROTEIN AND FAT

	PROTEIN	FAT
Carbon	53	76.5
Nitrogen	16	—
Hydrogen	7	12
Oxygen	23	11.5
Sulfur	1	—
	100	100

The data of Table 16 were obtained with a man on ordinary mixed diet.

TABLE 16. CALCULATION OF ENERGY METABOLISM FROM CARBON AND NITROGEN BALANCES. MAN OF 64 KILOGRAMS AT REST IN ATWATER RESPIRATION APPARATUS

	GRAMS PER DAY				
INTAKE	*Protein*	*Fat*	*Carbohydrate*	*Nitrogen*	*Carbon*
Total in food	94.4	82.5	289.8	15.1	239.0
Lost in digestion	5.4	3.7	3.2	0.9	7.4
Absorbed	89.0	78.8	286.6	14.2	231.6
OUTPUT					
By lungs					207.3
By kidneys				16.2	12.2
Metabolized				16.2	219.5
BALANCE				−2.0	+12.1

A loss of 2.0 grams of body nitrogen indicates ($2.0 \times 6.25 =$) 12.5 grams of body protein burned. Also there were 89.0 grams absorbed from food, and, therefore, in all, 101.5 grams of total protein burned.

The amount of carbohydrate burned was taken to be the same as that absorbed from the food, viz. 286.6 grams per day.

From the carbon balance and the above data, therefore, we estimate the amount of fat burned as follows:

12.5 grams body protein yield (12.5×53 per cent $=$)	6.6 grams carbon
and there were in the absorbed food	231.6 grams carbon
∴ total available was	238.2 grams carbon
But total catabolized was only	219.5 grams carbon
∴ the body stored in the form of fat	18.7 grams carbon

Since fat contains 76.5 per cent carbon, 1 gram carbon = 1.307 grams fat.
∴ 18.7 grams carbon = 24.4 grams fat.
The body therefore absorbed 78.8 grams fat
stored 24.4 grams fat
burned 54.4 grams fat

In all, the body burned per day

101.5 grams protein, yielding	(101.5×4.35 [a] $=$)	442 Calories
54.4 grams fat, yielding	(54.4×9.45 [a] $=$)	515 Calories
286.6 grams carbohydrate, yielding	(286.6×4.1 [a] $=$)	1175 Calories
		2132 Calories

[a] Here the factors for fuel value are not reduced to allow for loss in digestion, because this loss has already been deducted in computing the amount of each nutrient actually absorbed and rendered available.

By means of the carbon and nitrogen balance, Sonden and Tigerstedt studied the energy metabolism of eight resting men betwee

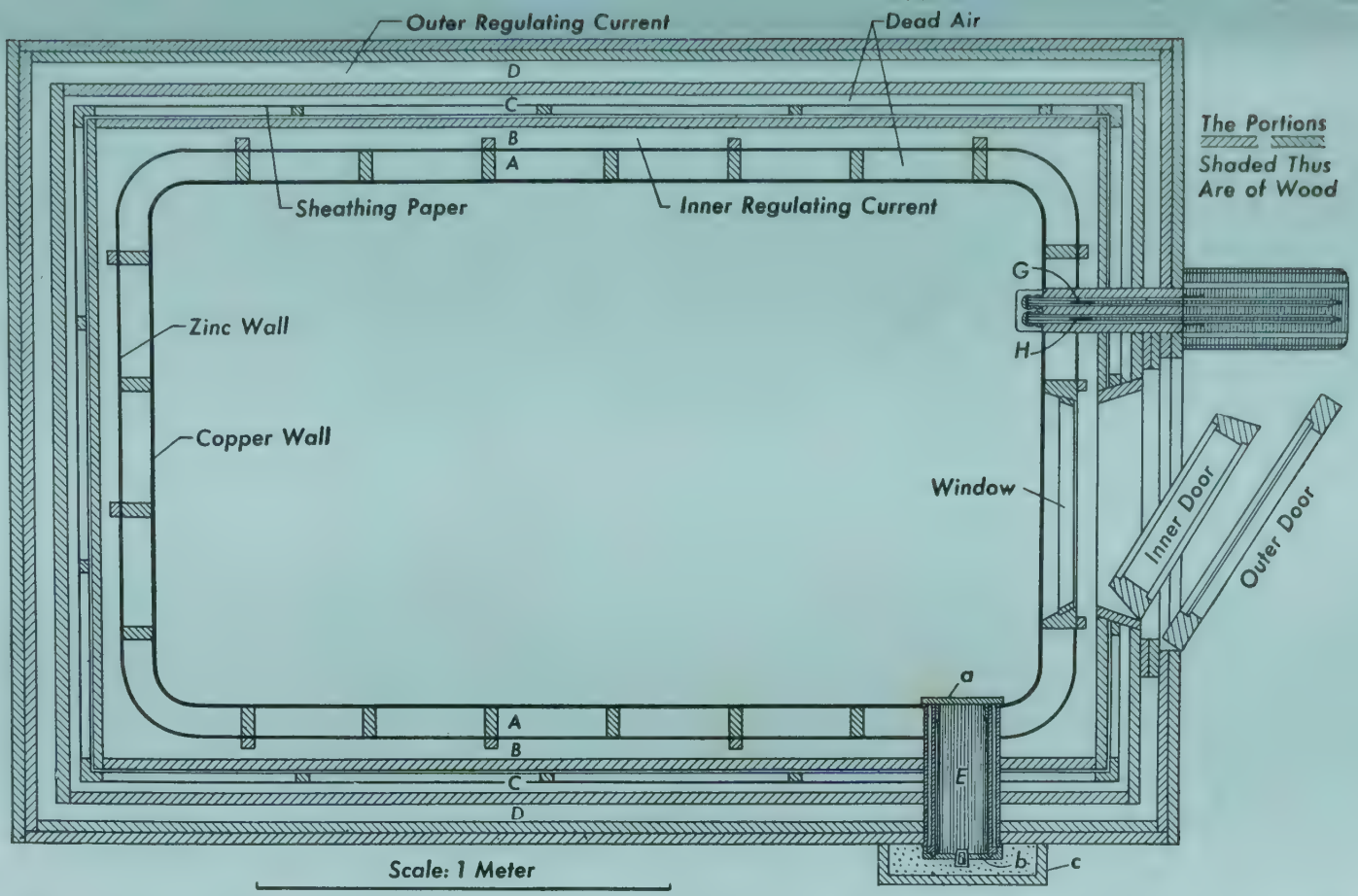

FIG. 6. Horizontal section of the original Atwater-Rosa-Benedict respiration calorimeter. (Courtesy of the United States Department of Agriculture.)

nineteen and forty-four years of age, with results which varied for the different subjects from 1853 to 2292 Calories per day. Many other experimenters have used the same method with similar results.

4. Calorimeter experiments. The most direct, and in some respects most convincing, way of ascertaining the energy metabolism is by the *method of direct calorimetry.* This consists in measuring the total energy expenditure of the body as heat or as heat and mechanical work by confining the subject in a chamber permitting of actual measurement of the heat produced. It was not until the development of the Atwater-Rosa-Benedict respiration calorimeter that complete and satisfactory data covering periods of one to several days were obtained. This apparatus consisted of an air-tight copper chamber, surrounded by zinc and wooden walls with air-spaces between, and was large enough for a man to live in without discomfort, being about 7 feet long, 4 feet wide, and 6½ feet high. An opening in the front of the apparatus, which was sealed with a pane of glass during an experiment, served as both door and window, admitting sufficient light for reading and writing. A smaller opening, having tightly fitting caps on both ends, was used for passing food, drink, excreta, etc., into and out of the chamber. The chamber was furnished with a folding bed, chair, and table, and was ventilated by means of a current of air, the carbon dioxide and water given off by the subject being removed by circulating the air through purifying apparatus, and the oxygen which the subject used being replaced by adding weighed amounts of oxygen to the air current as required. By this means it is possible to carry out, in the calorimeter, metabolism experiments in which the oxygen and hydrogen as well as the carbon and nitrogen balances are determined, and from these data the gain or loss of carbohydrate as well as of protein and fat can be measured.

The ventilating air current is so regulated that it enters and leaves the calorimeter at the same temperature; and between the copper and zinc walls are placed a large number of thermo-electric junctions connected with a delicate galvanometer by means of which each wall is tested every four minutes, day and night, during the progress of an experiment, and the mere traces of heat which may

pass to or from the calorimeter through its walls are quickly detected and made to balance each other. Thus there is no gain or loss of heat either through the walls of the chamber or by the ventilating air current, and the heat given off by the subject can leave only by the means especially provided for carrying it out and measuring it. A part of the heat liberated is carried from the chamber in latent form by the water vapor in the outgoing air, which is accurately

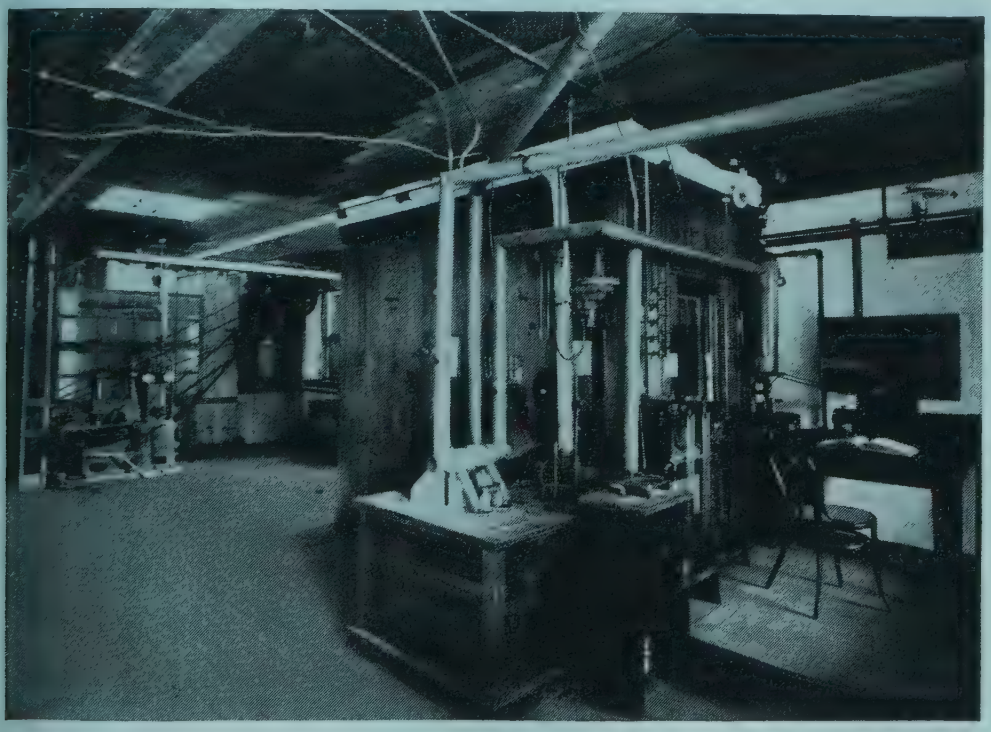

FIG. 7. The original Atwater-Rosa-Benedict respiration calorimeter. (Courtesy of the United States Department of Agriculture.)

determined. The rest of the heat is brought away by means of a current of cold water circulating through a copper pipe coiled near the ceiling of the chamber. The quantity of water which passes through the pipe and the difference between the temperature at which it enters and that at which it leaves the coil are carefully determined and show how much heat is thus brought out of the chamber.

In recent years several different forms of respiration apparatus

and calorimeters, based on the principles of the apparatus above described but adapted in size and shape to different types of experimentation, have come into use.

In 1913 Armsby compiled a summary of experiments upon men, dogs, and cattle which had been published up to that time and which showed that the difference between the total heat production

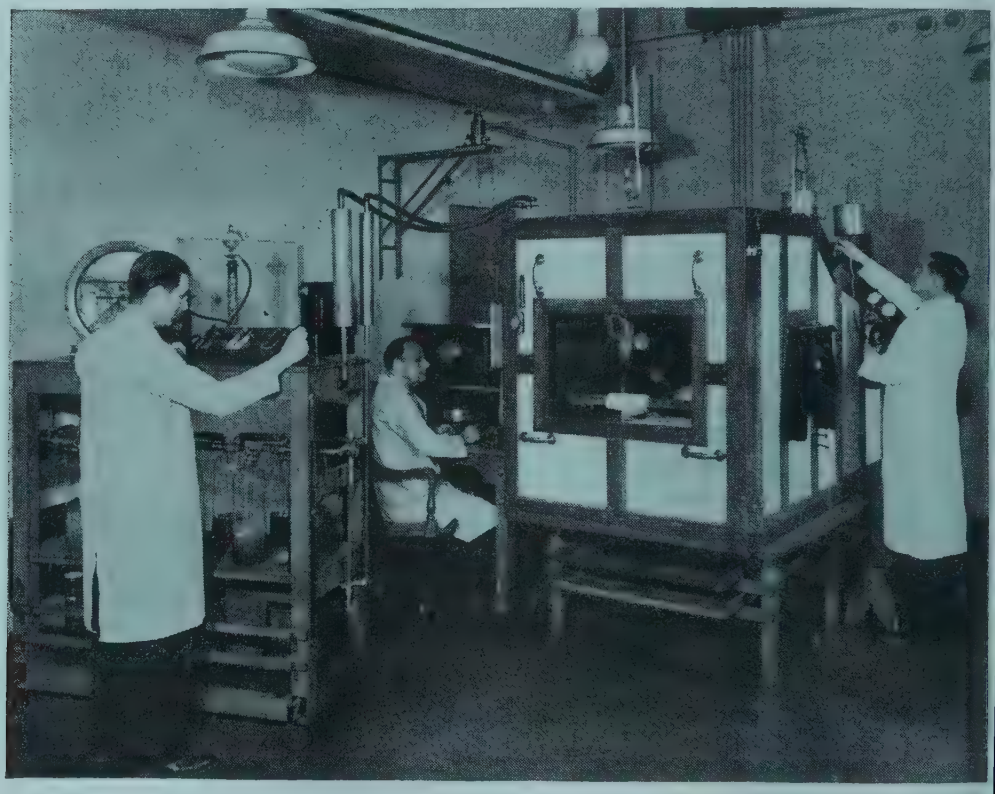

FIG. 8. The respiration calorimeter now in use at the Russell Sage Institute of Pathology. (Courtesy of Dr. E. F. DuBois.)

as computed and as directly measured was only one fourth of one per cent, or quite within the limits of accuracy of experimental methods of this sort.

Since the time of Armsby's compilation, the agreement between the observed and computed heat production has been confirmed in many additional experiments, both by the same and different experimenters. Three forms of apparatus are shown in Figures 7, 8, and 9

Summary of the Evidence Obtained by the Different Methods

A general view of the results obtained by all four of the methods described shows them to be strikingly consistent and leads to the conclusion that the food requirements of a young to middle-aged

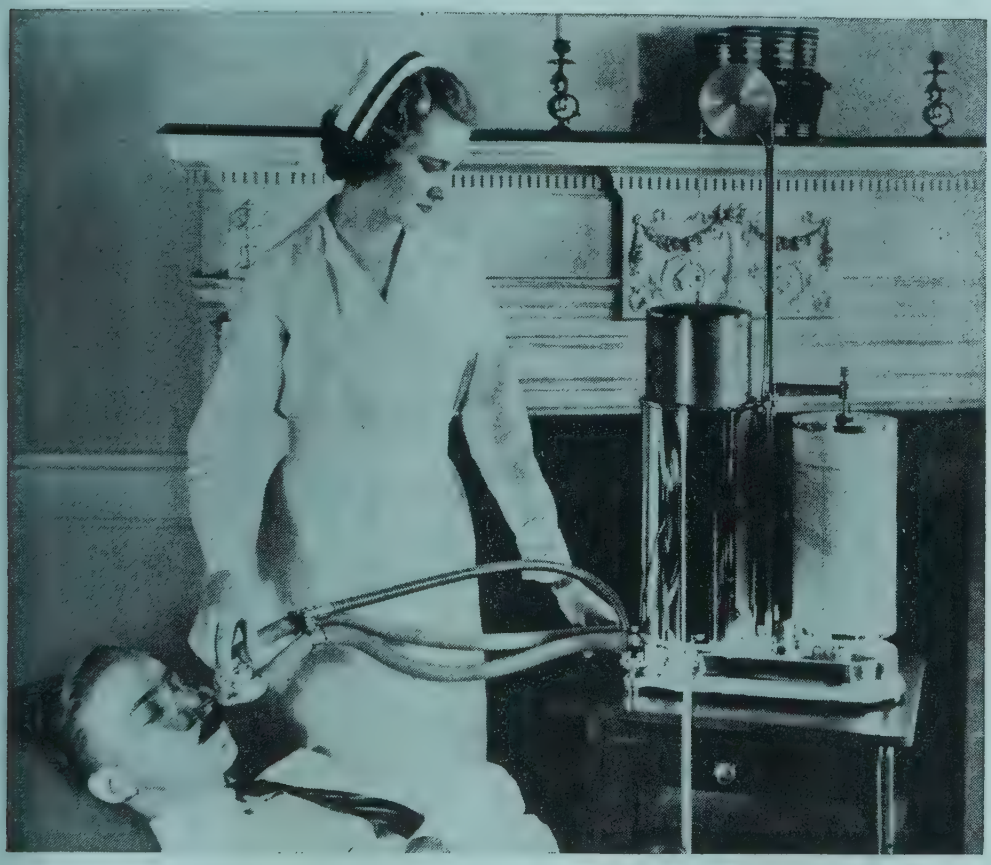

FIG. 9. Benedict-Roth portable respiration apparatus for determination of basal metabolism. (Courtesy of Warren E. Collins, Inc.)

man of average size, without muscular work, eating a mixed diet sufficient to meet his need, approximates 2000 Calories per day, and that such muscular activity as is incidental to very quiet living indoors may be expected to raise this requirement to about 2200 Calories per day.

Lusk summarized the mean energy requirement of an average-sized man in somewhat more precise terms as follows: Absolute rest in bed without food, 1680 Calories; Absolute rest in bed with food, 1840 Calories; Rest in bed 8 hours, sitting in chair 16 hours, with food, 2168 Calories.

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N.B.: See also the References and Suggested Readings at the end of Chapter 9.

Chapter 9

THE BASAL ENERGY METABOLISM, REGULATION OF BODY TEMPERATURE, AND SPECIFIC DYNAMIC ACTION

The term *basal metabolism* is used to designate the energy metabolism of the body when at complete rest (both mentally and physically) in the so-called "post-absorptive state" (12 to 18 hours after the last previous intake of food) in a room of comfortable temperature and when the body temperature is within normal range. "If this last condition is not satisfied, the deviation from the normal temperature must be determined." This basal energy metabolism for a given age and size is used as the starting point for the calculation of the total energy requirement of individuals of different muscular activities, and also as a basis of comparison in pathological disturbances, or in studies of the influence of age, sex, race, or of previous or immediate environment.

Such analyses as have been made of the maintenance requirement of the body, with reference to its principal functions, indicate that in the healthy adult the basal expenditure of energy may be attributed in part, perhaps about one third, to the functional activities of the various organs (heart action, kidney action, respiration, etc.). The greater part of the basal heat production is thought to be due to oxidations in the resting tissues, principally in maintaining the tone of the skeletal muscles. In the healthy adult the basal metabolism depends chiefly upon the intensity of these internal processes and the size, shape, and composition of the body.

Influence of internal activities. The work of maintaining the respiration and circulation obviously involves a continual expenditure of energy. It is clear too that deep and rapid breathing or vigorous heart action must involve an increased activity of the

muscles concerned. But it is not always clear to what extent increased respiratory and heart action are a cause, and to what extent they are an effect, of increased energy metabolism. Thus Murlin and Greer * emphasize the close relationship of the heart to the requirements of the tissues for energy in that the energy metabolism is immediately dependent upon oxygen supply. Since but little available oxygen can be stored in the living substance, "the response of the heart to variations in the (energy) requirement must be immediate and, within very narrow limits of time, proportional to this requirement."

A large factor in basal metabolism is the maintenance of muscular tension or tone. That every living muscle is always in a state of tension is evident from the fact that it gapes open if cut crosswise. It is equally evident that the degree of tension (and therefore the expenditure of energy required to maintain it) varies greatly in different individuals under similar conditions and in the same individual under different conditions. The differences observed by Atwater and Benedict between the metabolism of the sleeping hours and that of the hours spent sitting up without muscular movements (65 and 100 Calories an hour, respectively) are largely due to the more complete relaxation of the muscles during sleep. Thus there is in the "resting" muscle a continual expenditure of energy which first takes the form of muscular tension, or tone, but ultimately appears as heat, so that the heat production, or energy metabolism, of the body at rest depends to a considerable extent upon the degree of tension which still persists in the muscles.

Benedict and Carpenter reported the data cited in Table 17 for

TABLE 17. ENERGY METABOLISM DURING SLEEP: CALORIES PER HOUR

SUBJECT	SLEEP AFTER REST	SLEEP AFTER MODERATE WORK	SLEEP AFTER SEVERE WORK	SLEEP AFTER VERY SEVERE WORK
E.O.	69.3	74.8	—	—
J.F.S.	60.4	65.3	—	—
J.C.W.	77.2	—	83.1	—
B.F.D.	69.8	—	83.3	—
A.L.L.	78.3	—	83.7	97.9

* *American Journal of Physiology*, Vol. 33, page 253.

the energy metabolism during sleep (1 A.M. to 7 A.M.) following different conditions of activity and showing the after effects of work upon muscular tension during rest.

Influence of the size, shape, and composition of the body. For different sized adults of the same species, the *basal metabolism increases in proportion to the surface rather than the weight.* Thus Rubner collected the data shown in Table 18 from experiments upon seven different dogs, all full grown but differing greatly in size.

TABLE 18. RUBNER'S DATA ON ADULT DOGS OF DIFFERENT SIZES

NO.	BODY WEIGHT KILOGRAMS	HEAT PRODUCTION IN CALORIES PER DAY		
		Total	Per Kilogram of Body Weight	Per Square Meter of Body Surface
I	3.10	273.6	88.25	1214
II	6.44	417.3	64.79	1120
III	9.51	619.7	65.16	1183
IV	17.70	817.7	46.20	1097
V	19.20	880.7	45.87	1207
VI	23.71	970.0	40.91	1112
VII	30.66	1124.0	36.66	1046

Here the heat production in Calories per kilogram was over twice as great in the smallest as in the largest dog, but the total metabolism was nearly proportional to the surface area throughout.

That the relationship of basal metabolism to body surface is not due simply to loss of heat through the cooling effect of the environment will be apparent from the observations upon the regulation of body temperature.

Armsby, in his *Principles of Animal Nutrition,* cites the explanation offered by von Hösslin—that the internal work and the consequent heat production in the body are substantially proportional to the two-thirds power of its volume, and since the external surface bears the same ratio to the volume, a proportionality necessarily exists between heat production and surface.

Largely as the result of Rubner's work it became customary to express basal energy requirements in terms of surface rather than of weight; but on account of the difficulties involved in actual measurements of the surface it has usually been computed from the weight. The earlier computations were made by Meeh's formula.

$S = W^{2/3} \times C$, or $S = 12.3 \sqrt[3]{W^2}$, in which S represents surface, W the weight, and the constant 12.3 represents the average value found by Meeh in a series of measurements of men.

Lissauer's modification of Meeh's formula, based on measurements of infants, is used extensively for estimating the surface area of infants and young children:

$$S = 10.3 \sqrt[3]{W^2}.$$

Benedict and Talbot have modified the constant of this formula to adapt it to increases in body weight up to 40 kilograms.

A series of measurements of body surface made by DuBois and DuBois led them to the conclusion that Meeh's formula yields results higher than the true average. They used two methods for computing the surface: (1) from a series of nineteen measurements of different parts of the body, the surface of each part being computed and the results added together ("linear formula"), and (2) a "height-weight formula" which they derived mathematically from the data of all available measurements of height, weight, and surface.

The height-weight formula may be written thus:

$$A = W^{0.425} \times H^{0.725} \times C$$

or in the form:

$$\text{Log } A = (\text{Log } W \times 0.425) + (\text{Log } H \times 0.725) + 1.8564$$ in either of which

A = Surface area in square centimeters
H = Height in centimeters
W = Weight in kilograms
C = A constant (71.84).

Using this formula DuBois and DuBois have constructed a chart (Fig. 10) from which the approximate surface area in square meters may be obtained at a glance if height and weight are known. The data given in Table 19 have been taken from this chart.

Further mathematical discussion will be found on pages 133 and 135 of the third (1936) edition of DuBois' *Basal Metabolism in Health and Disease.*

On applying the height-weight formula to the recorded energy metabolism of the large number of men studied in Benedict's laboratory, as well as those in his own, DuBois finds that all the data for men under 50 years of age in these two series are within 15 per cent

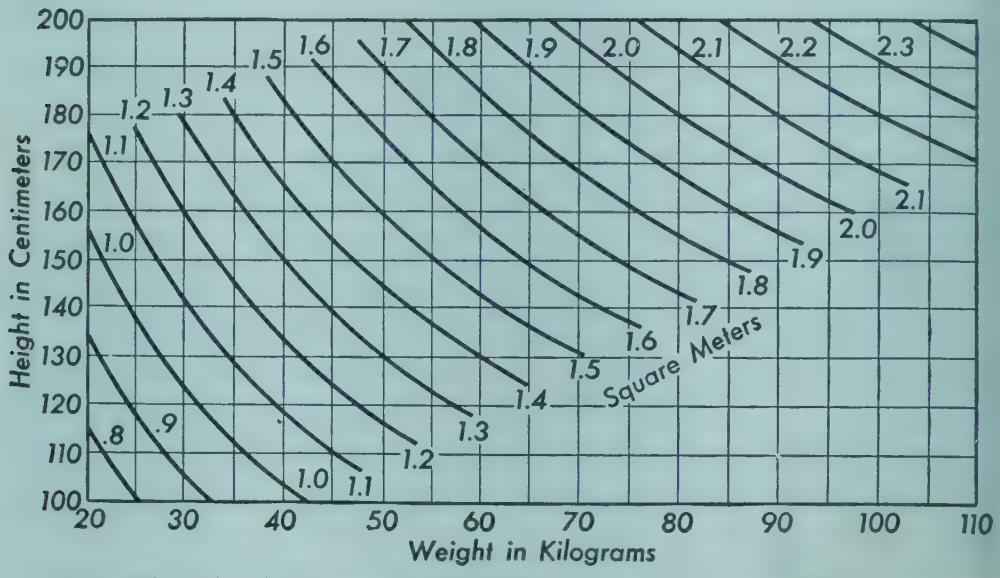

FIG. 10. Chart for determining surface area of man from weight in kilograms and height in centimeters according to DuBois formula. (Courtesy of Dr. E. F. DuBois.)

of the average basal heat production of 39.7 Calories per hour per square meter of surface area properly computed, and that 86 per cent of all the cases are within 10 per cent of his average. Means finds all of his 16 normal cases (9 men and 7 women) and also most of his obesity cases to fall within DuBois' "normal limits," i.e., within 10 per cent of DuBois' average of 39.7 Calories per hour per square meter.

Boyd (1935) has summarized the work on the determination of surface area in *The Growth of the Surface Area of the Human Body* to which the reader is referred for fuller treatment of the subject. Benedict (1938) in his monograph on *Vital Energetics* devotes a section to a critical examination of the relation between surface area and basal metabolism in man and other animals.

Differences of build (shape of body) are associated not only with varying ratios of weight to surface but also with differences of fat-

TABLE 19. SURFACE AREA IN SQUARE METERS FOR DIFFERENT HEIGHTS AND WEIGHTS

HEIGHT IN CENTIMETERS	WEIGHT IN KILOGRAMS																
	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100	105
200							1.84	1.91	1.97	2.03	2.09	2.15	2.21	2.26	2.31	2.36	2.41
195						1.73	1.80	1.87	1.93	1.99	2.05	2.11	2.17	2.22	2.27	2.32	2.37
190				1.56	1.63	1.70	1.77	1.84	1.90	1.96	2.02	2.08	2.13	2.18	2.23	2.28	2.33
185				1.53	1.60	1.67	1.74	1.80	1.86	1.92	1.98	2.04	2.09	2.14	2.19	2.24	2.29
180				1.49	1.57	1.64	1.71	1.77	1.83	1.89	1.95	2.00	2.05	2.10	2.15	2.20	2.25
175	1.19	1.28	1.36	1.46	1.53	1.60	1.67	1.73	1.79	1.85	1.91	1.96	2.01	2.06	2.11	2.16	2.21
170	1.17	1.26	1.34	1.43	1.50	1.57	1.63	1.69	1.75	1.81	1.86	1.91	1.96	2.01	2.06	2.11	
165	1.14	1.23	1.31	1.40	1.47	1.54	1.60	1.66	1.72	1.78	1.83	1.88	1.93	1.98	2.03	2.07	
160	1.12	1.21	1.29	1.37	1.44	1.50	1.56	1.62	1.68	1.73	1.78	1.83	1.88	1.93	1.98		
155	1.09	1.18	1.26	1.33	1.40	1.46	1.52	1.58	1.64	1.69	1.74	1.79	1.84	1.89			
150	1.06	1.15	1.23	1.30	1.36	1.42	1.48	1.54	1.60	1.65	1.70	1.75	1.80				
145	1.03	1.12	1.20	1.27	1.33	1.39	1.45	1.51	1.56	1.61	1.66	1.71					
140	1.00	1.09	1.17	1.24	1.30	1.36	1.42	1.47	1.52	1.57							
135	0.97	1.06	1.14	1.20	1.26	1.32	1.38	1.43	1.48								
130	0.95	1.04	1.11	1.17	1.23	1.29	1.35	1.40									
125	0.93	1.01	1.08	1.14	1.20	1.26	1.31	1.36									
120	0.91	0.98	1.04	1.10	1.16	1.22	1.27										

ness, i.e., of body composition. The thin man, besides having a greater surface in proportion to his weight, differs also from the stout man in that a larger percentage of his body is active tissue (actual protoplasm). Since the metabolism of the body depends more upon its weight of protoplasm (active tissue) than upon its total weight, we have here an important reason for believing that the food requirement will be greater in a tall, thin man than in a shorter and fatter man of the same weight.

Even with the same height and weight there may be differences in the composition of the body. Thus a man of average height and weight but large-boned and loosely built will be of less than average fatness; a man of the same height but less broad-shouldered must be somewhat fatter in order to weigh the same. Hence equality of height and weight does not necessarily imply the same shape and composition of body. Benedict finds that among normal adults of like height and weight the basal metabolism of athletes is about 5 per cent higher, and that of women about 5 per cent lower, than that of average non-athletic men. He attributes these divergencies to differences in body composition, holding that women have somewhat more fat, and athletes somewhat less, than non-athletic men of the same weight and height.

Standards for normal basal metabolism. On the basis of the data from many determinations, tables or formulas for predicting the basal metabolism of normal individuals have been evolved by Aub and DuBois, by Harris and Benedict, by Dreyer, and by Boothby, Berkson, and Dunn.

TABLE 20. "SAGE NORMAL STANDARDS" OF AUB AND DUBOIS

AGE IN YEARS	CAL. PER SQ. M. OF BODY SURFACE PER HOUR	
	Males	Females
14–16	46.0	43.0
16–18	43.0	40.0
18–20	41.0	38.0
20–30	39.5	37.0
30–40	39.5	36.5
40–50	38.5	36.0
50–60	37.5	35.0
60–70	36.5	34.0
70–80	35.5	33.0

The figures of Aub and DuBois are most significant for the age range where the greatest number of studies have been made (males from 20 to 40 years). The figures for females are not the averages of direct determinations but were calculated from an examination by Gephart and DuBois of the results of studies of the basal metabolism of women by Benedict and Emmes and by Means as 7 per cent below the average for males.

Krogh states that these Aub-DuBois standards are too high and has published a table of modified Aub-DuBois standards for the ages of 15 to 75 years with a uniform reduction of 6 per cent.*

Harris and Benedict made a biometric study of their findings on 136 men, 103 women, and 94 infants in an attempt to evolve an accurate method of calculating unknown basal metabolism. The prediction formulas they derived are as follows:

For males: $H = 66.473 + 13.752\,W + 5.003\,S - 6.755\,A$

For women: $H = 655.096 + 9.563\,W + 1.850\,S - 4.676\,A$

H = Total heat production in 24 hours

W = Weight in kilograms

S = Stature in centimeters

A = Age in years.

(The fact that each of these formulas is given in the literal terms of its derivation may make the comparison of them somewhat disconcerting at first glance. On working through the two formulas, however, it will be seen that there is neither a mistake of decimal point nor a great difference between the basal metabolisms of the sexes.)

Calculation of results by these formulas is much simplified by the use of tables compiled by Carpenter.†

Krogh holds that the Harris and Benedict formulas predict accurately only for individuals within the range of weight, height, and age from which these measurements were taken. He believes that

* Krogh's *Recording Respiration Apparatus—Tables of Normal Metabolic Rates.* J. H. Schultz, Copenhagen, 1925.

† Carpenter, *Tables, Factors and Formulas for Computing Respiratory Exchange and Biological Transformations of Energy.* Carnegie Institution of Washington, Publication No. 303 A (1924).

they do not hold good for individuals whose weight varies widely from the average for their height and age.

Dreyer in 1920 made a statistical study of the data of Harris and Benedict, with the object of evolving formulas which would predict basal metabolism more accurately than those of Harris and Benedict or the standards of Aub and DuBois. These formulas, which are applicable to males and females of the age of 5 years upward, are as follows:

For males: $$C = \frac{\sqrt{W}}{0.1015 \times A^{0.1333}}$$

For females: $$C = \frac{\sqrt{W}}{0.1127 \times A^{0.1333}}$$

C = Calories per 24 hours
W = Weight in grams
A = Age in years.

The average basal metabolism of 17 normal college women studied by Blunt and Dye was 6.5 per cent below the Aub-DuBois and 4.1 per cent below the Harris-Benedict standards.

MacLeod and Rose found the average deviation of 92 normal women between the ages of 20 and 50 to be 8.6, 4.5, and 3.4 per cent below the Aub-DuBois, Harris-Benedict, and Dreyer predictions respectively.

Boothby and Sandiford found the Aub-DuBois standards 1 to 4 per cent high for adults and 3 to 7 per cent high for children and on the basis of these findings have published a table of modified Aub-DuBois standards. Boothby, Berkson, and Dunn (1936) after statistical analysis of still more data proposed another set of standards in Calories per square meter per hour to be known as the Mayo Foundation Standards. Benedict in a study of 27 normal men and 33 normal women found that the basal metabolism of the men averaged 4.4 per cent below the Aub-DuBois and 1.8 per cent below the Harris-Benedict predictions, while that of the women averaged 7.3 and 4.2 per cent below the same standards respectively.

McKay found the basal metabolism of a group of women 35 to 50 years of age to average about 7.3 per cent below the Aub-DuBois standards for women of this age range, while that of a group 50 to

60 years of age showed an average deviation of − 11.4 per cent. Jenkins has reported that analysis of the results on 2994 women and 1126 men gave average deviations of − 7.5 per cent, − 6.6 per cent, and − 6.8 per cent from the Aub-DuBois, Harris-Benedict, and Dreyer predictions respectively. Stark, in studies of the basal metabolism of a large group of young men and women 17 to 21 years of age, reaches the conclusion that the best standards for this age range are those obtained from a straight-line extrapolation of the Harris-Benedict adult standards.

These findings seem quite consistent in indicating that for women the Aub-DuBois standards may be 7 to 8 per cent too high and those of Harris and Benedict, 4 to 5 per cent too high, while for men both standards may be about 4.5 per cent too high.

Standards for children. Standards for basal metabolism in children are still a matter of research. The Aub-DuBois standards do not extend below the age of 14 years and for girls of the ages to which they apply seem to be too high. Harris and Benedict give no figures for ages below 21 years in the prediction tables based on their formulas but experience with these formulas indicates that although they do not predict accurately for girls they seem to apply well to boys. The Dreyer formulas are applicable to both boys and girls 5 years of age and above and in general give results agreeing well with those obtained in practice on normal children. For boys weighing 3 to 38 kilograms Benedict and Talbot have established standards based on body weight.

For girls from 1 week to 12 years of age Benedict has proposed predicting the basal metabolism from height. A frequent difficulty in using these standards is that many 11- and 12-year-old girls are taller than the maximum height given in the table, while for the younger girls whose heights occur in the table the predictions seem to be somewhat low. On the basis of his work with groups of Girl Scouts in his respiration chamber Benedict has set up standards for girls 12 to 20 years of age predicting the basal heat production for each half-year of age, but these figures having been obtained during sleep are found to be too low when compared with actual basal metabolisms obtained under the usual standard conditions.

Talbot (F. B.) has proposed standards for boys and girls on the basis of body weight, giving the basal metabolism in Calories per 24 hours while Talbot (N. B.), Worcester, and Stewart have proposed predicting the basal metabolism of children from creatinine output.

In studies of the basal metabolism of 3- and 4-year-old children (17 girls and 12 boys) Robb found that the girls averaged 25 per cent above the Benedict standard based on height, and 9 per cent below the Dreyer standard, while the average deviation of the boys from the Benedict and Talbot standard (based on weight) was + 19 per cent, from the Dreyer standard − 13 per cent, and from the Harris-Benedict standard + 17 per cent.

Williams, studying a smaller group of children of this age range, found similar deviations: for girls + 26 per cent from the Benedict (height) standard and − 10 per cent from the Dreyer standard; while for the boys the deviations were + 20 per cent from the Benedict and Talbot standard based on weight, − 13 per cent from the Dreyer, and + 19 per cent from the Harris-Benedict standard.

Robertson, working with children 6 to 8 years of age (8 girls and 8 boys), reported average deviations for the girls of −2.8, −5.5, −2.0, + 4.9, and + 9.0; for the boys of − 8.5, + 9.8, − 3.4, + 5.8, and + 11.3 from the Dreyer, the Harris and Benedict, the Mayo, the Lewis *et al.* (area and age), and the Talbot (weight) standards respectively.

Working with 9-year-old girls Potgieter found average deviations of + 9.1 per cent and − 5.8 per cent from the Benedict height and the Dreyer predictions respectively, while in a group of girls 10 to 12 years of age Thompson found average deviations of − 1.6 and − 8.5 per cent from these same standards. Taylor, working with 9- to 11-year-old boys, reported average deviations of − 10.1, + 4.3, and + 4.6 per cent from the Dreyer, the Benedict and Talbot, and the Harris and Benedict standards respectively. Lamb, working with boys 12 to 15 years of age, reported average deviations of − 7.1, + 0.9, and − 11.4 per cent from the Dreyer, the Harris and Benedict, and the Mayo standards respectively. For infants Levine and Marples have found the Benedict and Talbot standards satisfactory. The best procedure at the present time seems to be that of comparing

the actual basal metabolism of a child with all the standards which can be applied before saying whether or not it is within normal range.

Investigators have found variations of 10 to 15 per cent from the average basal metabolism in normal individuals of the same age group. Differences of similar magnitude are found in determinations made on the same individual over a prolonged period.

Influence of brain and nerves. In any consideration of this question it is important to distinguish sharply between the nervous control of muscular conditions and the metabolism of the brain and nerve substance itself. As emphasized particularly by Mathews, the brain receives a copious blood supply, and the blood coming to the brain is arterial, while that leaving the brain is venous, indicating that considerable oxidative metabolism occurs in brain tissue. Tashiro and others have shown that the carbon dioxide production of nerve fiber is increased when the nerve is stimulated to activity. But since the entire weight of brain and nerve substance constitutes only about 2 per cent of the body weight, it remains questionable whether, even if its metabolism increases with "mental activity," the increase would be appreciable in measurements of the energy expenditure of the body as a whole. Among the best-controlled experiments upon this problem of energy expenditure are those of Benedict and Carpenter, in which 22 college students were given their mid-year examinations in the respiration calorimeter and their energy metabolism during the three-hour period covered by the examination was compared with that during the same period on another day when the student sat in the calorimeter at rest. In some individuals the metabolism was higher during the examination period, while in others it was lower—results much more likely due to involuntary increase or decrease of muscular tension than to altered metabolism of the brain tissue. In the average of the entire series of experiments there appeared a slight increase of oxygen consumption, carbon dioxide output, and heat production during the examination, but the increase was so small and the exceptions so numerous that the investigators were not willing to conclude from their results that mental work has any positive effect upon

the total metabolism, but rather infer the opposite. More recent experiments by Benedict and Benedict with apparatus permitting shorter periods and more exact measurements have shown that intense mental effort increases energy expenditure about 4 per cent above the basal rate, an amount quite insignificant as compared with the increases due to ordinary muscular activities.

Apparently we must conclude that such changes in energy metabolism as may result from differences in activity of the brain and nerves involved in the performance of mental work are so small, in comparison with the energy exchanges always going on in the muscles, that the former are quite obscured by the unavoidable fluctuations of the latter, and so play no measurable part in determining the total food requirement of the body. This conclusion, however, does not exclude the probability of stimulation of the basal rate by strong emotions, acting directly to increase muscle tone or through the mechanism of the internal secretions in ways such as those suggested in the following paragraph.

Influence of internal secretions and pathological conditions. Some internal secretions influence the basal metabolism, notably that of the thyroid gland, thyroxine, which is quite specifically a regulator of the rate of oxidation in the body. If for any reason the thyroid gland becomes overactive, the basal metabolism increases. This increase has been known to amount to as much as 75 per cent. If, on the other hand, the activity of the thyroid is reduced, a lowering of the basal metabolism, which may amount to as much as 30 per cent, results. Another internal secretion which affects the basal metabolism is adrenaline (adrenine, epinephrine), but its effect is much less and of much shorter duration than that of thyroxine. Overactivity of the pituitary gland tends to result in a rise of the basal metabolism, while subnormal activity may cause a fall in the basal rate. These changes are not usually of very great magnitude and are not nearly as characteristic as those due to abnormal functioning of the thyroid gland and the adrenals.

Certain pathological conditions are accompanied by a characteristic alteration in the basal rate. DuBois reports an average increase in fevers of 13 per cent for each degree Centigrade (or 7.2 per cent

for each degree Fahrenheit) rise in temperature above the normal level. Basal metabolism is accelerated in exophthalmic goiter, leukemia, malarial and typhoid fevers, and in tuberculosis accompanied by fever; it is neither increased nor decreased materially in adolescent goiter, tuberculosis unaccompanied by fever, uncomplicated cases of obesity, arthritis, gout, nephritis, and cardiac and renal disorders. Hypothyroidism and myxedema are marked by a lowered heat output. For a more extended discussion of the clinical significance of basal metabolism determinations, see DuBois, *Basal Metabolism in Health and Disease.*

Influence of age and growth. From the fact that in animals of the same species, but of different size, the heat production is proportional to the surface rather than to the weight it would follow that children must have a greater maintenance requirement per unit of weight than adults.

From the data obtained in extensive studies of the basal metabolism of children, Benedict and Talbot have made charts showing the results in individual cases with curves indicating the averages.

The new-born infant has an average basal metabolic rate of 48 Calories per kilogram per 24 hours, much lower than the rate for older children. Krogh attributes the regular increase in basal metabolism in infancy to "the development of the muscular system as such and perhaps simply the gradual development of muscle tone." Since the rate is comparatively low during the first few months when growth is most rapid, the high metabolism of children does not seem to be due to a specific stimulus associated with growth.

There is some question whether the downward trend of the basal metabolic curve after the second year is temporarily broken during the prepubescent period. Comparative studies of boys made by DuBois before and after the onset of puberty indicate that there is a temporary rise in the prepubescent period. Benedict and Hendry, in group determinations on 105 girls, found no proof of the influence of prepubescence on the metabolic rate. MacLeod, in 362 experiments upon 43 girls between 11 and 14 years of age, obtained results which indicate that girls of 12 and 13 show an increased rate

of metabolism corresponding to that shown by the boys of the DuBois studies.

The average results of comparable data here shown in Table 21 are taken, by kind permission of the authors, from MacLeod and Taylor's revision of *Rose's Foundations of Nutrition*. From these data the student may construct comparative curves for boys and girls of 1 to 18 years of age.

TABLE 21. BASAL ENERGY METABOLISM OF CHILDREN

AGE IN YEARS	CALORIES PER KILOGRAM BODY-WEIGHT PER HOUR	
	Boys	Girls
1	2.33	2.33
2	2.29	2.29
3	2.13	2.00
4	1.96	1.83
5	1.88	1.75
6	1.79	1.67
7	1.71	1.63
8	1.67	1.58
9	1.58	1.54
10	1.54	1.50
11	1.46	1.42
12	1.42	1.33
13	1.67	1.29
14	1.71	1.54
15	1.50	1.33
16	1.38	1.25
17	1.25	1.17
18	1.25	1.08

The metabolic rate decreases throughout adult life. The da are still insufficient to permit a definite statement as to the ra of decrease in different age periods; and no doubt individual d ferences are relatively large.

Benedict found the basal metabolism of a woman studied betwe the ages of 24 and 36 years remarkably constant, while two m studied between the ages of 30 and 57 years showed a distinct crease as age increased, the most marked change appearing at age of 47 in the one, of 42 in the other. In a third man, studied tween the ages of 43 and 59, the metabolism remained essenti constant, due, perhaps, Benedict suggests, to the effect of age be offset by increase in body weight and improvement in phys condition.

Data on *elderly people* (60 years and over) have accumulated slowly. Reports have now been made on about 100 men in this age range by Benedict, by Lewis, by Hitchcock and Matson, by Boothby, Berkson, and Dunn, by Harris and Benedict, and by Aub and DuBois; and on about 75 women by Benedict and Meyer, by Hitchcock and Matson, by Boothby, Berkson and Dunn, by Harris and Benedict, and by McKay and Patton. These results indicate that the basal metabolism decreases slightly as age advances; but more studies are needed at all ages between 60 and 100 years before satisfactory standards can be set up for this age range.

Comparison of the sexes. Benedict and Talbot found no difference in the basal metabolic rate of boys and girls of the same age until they reach a weight of 11 kilograms or a surface area of 0.48 square meter. Beyond a weight of 14 kilograms, boys maintain a persistently higher rate which is not apparently due at this early age (about 3 years) to greater muscular development.

Influence of sleep. Reports by Benedict, Benedict and Carpenter, and Mason and Benedict on the effect of sleep on the basal metabolism of adults are in fairly close agreement, indicating an average drop below the waking metabolism of about 10 per cent during sleep.

Summary. In a general review of the factors affecting normal basal metabolism Benedict concluded that "the basal metabolism of an individual is a function, first, of the total mass of active protoplasmic tissue, and, second, of the stimulus to cellular activity existing at the time the measurement of the metabolism was made." And that: "Perhaps the most striking factors causing variations in the stimulus to cellular activity are age, sleep, prolonged fasting, character of the diet, and the after effect of severe muscular work."

Regulation of Body Temperature *

Climate, season, housing, clothing, are all factors which may influence energy metabolism principally through their bearing upon

* For full discussion of the influence of surrounding temperatures upon metabolism and the relation of metabolism to the regulation of body temperature the reader is referred to Lusk's *Science of Nutrition.*

the regulation of body temperature. It is evident that the maintenance of the body at a temperature above that of its ordinary environment involves a continual output of heat. This output of heat may be regulated in either of two ways: (1) By variations in the quantity of blood brought to the skin, which tend to control the loss of heat by radiation, conduction, and sweating; this is called "physical regulation." (2) By an increase in the rate of oxidation in the body in response to the stimulus of external cold; such a change in the rate of oxidation is known as "chemical regulation." The extra heat production which follows the taking of food (the specific dynamic action of the foodstuffs) may take the place of the "chemical regulation" and so help to protect the body from the necessity of burning material simply for the maintenance of its temperature. Muscular work, by increasing the production of heat in the body, may also render chemical regulation unnecessary; but apparently the specific dynamic action does not furnish energy which can be utilized for muscular work.

The presence of a layer of adipose tissue under the skin as well as the custom of covering the greater part of the external surface with clothing also tends to keep down the loss of heat to the point where "physical regulation" will suffice. Lusk cites experiments by Rubner upon a man whose metabolism was determined when kept in the same cold room but with different amounts of clothing, and observed that when the man was sufficiently clothed to be comfortable the "chemical regulation" was eliminated (*Science of Nutrition*, 4th edition, page 163).

In general it seems probable that people warmly clothed and living in houses which are heated in winter are not called upon to exercise "chemical regulation" to any considerable extent, the heat required for the maintenance of body temperature being obtained in connection with the metabolism which is essential to the maintenance of the muscular tension and the various other forms of internal work. If, however, the body be exposed to cold, it may be forced to employ "chemical regulation" with a resulting increase of the food requirement, and this will occur more readily in a thin person than in one who is well protected by subcutaneous fat.

The extra heat required in cold weather is probably obtained mainly through the activities of the muscles. It is a matter of general experience that one instinctively exercises the muscles more vigorously in cold weather than in warm, and if one attempts to endure much cold without muscular exercise there results shivering —a peculiar involuntary form of muscular activity whose function appears to be to increase heat production.

To a large extent therefore the regulation of body temperature, in case of exposure to cold, is accomplished through the activity and tension of the muscles.

The foregoing discussion has reference primarily to adults. In the case of the infant whose surface is much greater in proportion to his weight and whose muscle tone is not yet fully developed, the loss of heat to the surroundings is not so readily checked by "physical" nor so easily made good by "chemical" regulation. Unless the infant is either warmly clothed or supplied with an artificial source of heat in cold weather he may be forced to burn, for warmth, material that might better be employed for growth.

The Specific Dynamic Action of Foodstuffs

Atwater and Benedict determined directly by means of the respiration calorimeter the heat production of the same man during five fasting experiments of one to two days each, and during a four-day experiment with food about sufficient for maintenance. The average total metabolism on the fasting days was about 9 per cent lower than on the days when food was taken.

Rubner found that each type of food exerted a more or less specific influence upon the energy metabolism, so that when the foodstuffs were fed separately, somewhat different energy values were required for the maintenance of body equilibrium. Thus, if the total metabolism of a dog fasting at 33° C. be represented by 100 Calories, he must be fed, in order to prevent loss of body substance, about 106.5 Calories of sugar, or 114.5 Calories of fat, or 140 Calories of protein. Lusk, however, has reported as average, typical results of experiments with dogs in his laboratory, 106 Calories of glucose, 104 Calories of fat, or 130 Calories of meat protein, indicating that

the specific dynamic action of fat is less than that of carbohydrate rather than more as Rubner reported. A man observed by Rubner metabolized in fasting 2042 Calories; when fed 2450 Calories in the form of sugar alone, he metabolized 2087 Calories; when fed 2450 Calories in the form of meat alone, he metabolized 2566 Calories.

Lusk and his coworkers investigated this influence of the foodstuffs upon metabolism, called the specific dynamic action, very extensively and developed the subject to such an extent that for an adequate discussion of their results the original articles in the *Journal of Biological Chemistry* or Lusk's own summaries * should be consulted. The adjustment of total food energy intake to the activities of everyday life is considered in the next chapter.

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Chapter 10

TOTAL ENERGY METABOLISM AND FOOD REQUIREMENT

In Chapters 8 and 9 we have considered the general aspects of energy needs in human nutrition and of the energy values of foods in meeting these needs. We saw in Chapter 8 that four types of investigation have been used to ascertain the rate at which our bodies "spend" (transform) energy according to their age, size, and activities. And in Chapter 9 we have studied somewhat fully the quantitative question of the rate of energy metabolism under the "basal" conditions of freedom from any immediate influence of food consumption or muscular activity.

Influence of Muscular Work

Muscular work is much the most prominent of the factors which raise the food requirements of adults above the basal rate necessary for mere maintenance.

Accurate measurements by means of the man-size respiration calorimeter have shown that the average total metabolism of a man sitting still is about 100 Calories per hour; while the same man working actively with the large muscles tends to increase his metabolism up to perhaps about 300 Calories per hour; and a well-trained man working with his largest muscles at maximum capacity may metabolize material enough to liberate up to 600 Calories per hour. That is, his metabolism may be six times as active during the hours actually spent in such heavy work as when he is at rest. If during 24 hours a man works as hard as this for 8 hours and spends 2 hours in such light exercise as going to and from work, his food requirement for the day will be somewhat over 6000 Calories, or three times the maintenance requirement. Thus, work may increase the day's

metabolism as much as 200 per cent, whereas liberal feeding at the end of a fast was found to increase the energy metabolism only about 22 per cent, or one ninth as much. Only a few exceptional occupations, such as that of lumbermen, for example, involve such heavy work as to cause a metabolism of 6000 Calories per day. More often the man who works eight hours a day at manual labor will increase his metabolism by 1000 to 2000 Calories above what is needed for maintenance at rest, making his total food requirement 3000 to 4000 Calories. The importance of muscular activity, as the chief factor governing the energy expenditure and food requirement of healthy adults, calls for a careful quantitative study of its effect upon metabolism.

Quantitative relation between work performed and total metabolism. Theoretically it is possible to determine the mechanical efficiency of a man by dividing the mechanical effect of his work by the increase of energy metabolism which the work involves. This gives the basis on which to ascertain how much extra food would be necessary to supply the energy required for the performance of any given task.

Benedict and others have studied the mechanical efficiency of both men and women in level and grade walking. These studies indicate that in level walking the efficiency is highest at moderate speeds. Very fast walking (about 5½ miles per hour) required a greater heat output than running at the same speed. In grade walking the expenditure for a given amount of work tended to be less at low speed on a high grade than at high speed on a low grade.

By means of the respiration calorimeter, Atwater and Benedict studied the question of mechanical efficiency with a different form of work that also used the large muscles. They placed in the calorimeter chamber an ergometer, which consisted of a fixed bicycle frame having in place of the rear wheel a metal disk which revolved against a measured amount of electrical resistance, so that the mechanical effect of the muscular work was very accurately determined. The expenditure of energy involved in the performance of this work was estimated by comparing the total metabolism of a working day with that of the same man when living in the calorimeter chamber

at rest. The average results obtained with three different men were as shown in Table 22.

TABLE 22. ATWATER AND BENEDICT DATA ON MECHANICAL EFFICIENCY OF MAN

SUBJECT AND NATURE OF EXPERIMENT	ENERGY TRANSFORMED		HEAT EQUIVALENT OF WORK PERFORMED	MECHANICAL EFFICIENCY
	Total per Day	Excess over That at Rest		
	Calories	*Calories*	*Calories*	*Per Cent*
Subject E. O.				
Average 13 rest experiments (42 days)	2279			
Average 3 work experiments (12 days)	3892	1613	214	13.3
Subject J. F. S.				
Average 4 rest experiments (12 days)	2119			
Average 6 work experiments (18 days)	3559	1440	233	16.2
Subject J. C. W.				
Average 1 rest experiment (4 days)	2357			
Average 14 work experiments (46 days)	5143	2786	546	19.6

With an improved ergometer of the same type as that used in the experiments just cited, Benedict and Carpenter, working with J. C. W. (one of the 3 men just mentioned), found efficiencies ranging from 20.7 to 22.1 per cent and averaging 21.6 per cent; with other men studied, the efficiencies ranged from 18.1 to 21.3 per cent.

Only under the most favorable circumstances, and with subjects fully accustomed to the kind of work being performed, will the actual mechanical effect produced amount to as much as one fourth to one third of the extra energy expended during work over that during rest, i. e., to an efficiency of 25 to 33 per cent. Not only do most occupations involve kinds of work which in their nature mu

be done with less efficiency than walking (or riding a stationary ergometer), but also, the usual hours of labor are longer than those in which the maximum mechanical efficiency is attained. The efficiency may begin to decline before any sensation of fatigue is felt.

Thus Leo Zuntz found, when he rode his bicycle for four successive hours at an average rate of 15 to 17 kilometers (about 9 miles) per hour, that he experienced no feeling of fatigue, but his determinations showed that the expenditure of energy necessary to produce a given effect had increased about 9, 13, 10, and 23 per cent at the end of 1, 2, 3, and 4 hours respectively. This is because, if the same kind of work be performed for a series of hours, auxiliary muscles are gradually brought increasingly into action, partly for the performance of the work itself, partly for the fixation of the bodily framework (maintenance of posture). These auxiliary muscles work less economically than those which are used first and most naturally. For much the same reasons there is a lower efficiency in the case of work which is from the first of too fatiguing a nature because of being either excessive or unsuitably distributed. When, in the work just mentioned, Leo Zuntz increased his speed 2.4 times, he found his metabolism increased 4.3 times, implying a considerably lowered efficiency. Under the conditions of Benedict and Cathcart's experiments also, the efficiency was usually decreased upon increasing the speed. On the other hand, a moderately heavy load was more economical than a light one.

MacLeod and associates have studied the mechanical efficiency of children from 6 to 15 years of age and have found the gross mechanical efficiency to range between 12 and 16 per cent, and the net efficiency between 23 and 32 per cent.

Total Energy Requirement of Adults

It is now possible to estimate the approximate average expenditure of energy per hour under a considerable number of conditions of muscular activity. For convenience of comparison and application the original data have been reduced to a common body-weight basis of 70 kilograms (154 pounds), with the results shown in Table 23, compiled by the late Professor Mary S. Rose.

TABLE 23. TOTAL ENERGY EXPENDITURE PER HOUR UNDER DIFFERENT CONDITIONS OF MUSCULAR ACTIVITY

FORM OF ACTIVITY	CALORIES PER HOUR		
	Per 70 Kilograms (Average Man)	*Per Kilogram*	*Per Pound*
Sleeping	65	0.93	0.43
Awake lying still	77	1.10	0.50
Sitting at rest	100	1.43	0.65
Reading aloud	105	1.50	0.69
Standing relaxed	105	1.50	0.69
Hand sewing	111	1.59	0.72
Standing at attention	115	1.63	0.74
Knitting (23 stitches per minute on sweater)	116	1.66	0.75
Dressing and undressing	118	1.69	0.77
Singing	122	1.74	0.79
Tailoring	135	1.93	0.88
Typewriting rapidly	140	2.00	0.91
Ironing (with five-pound iron)	144	2.06	0.93
Dishwashing (plates, bowls, cups, and saucers)	144	2.06	0.93
Sweeping bare floor (38 strokes per minute)	169	2.41	1.09
Bookbinding	170	2.43	1.10
"Light exercise"	170	2.43	1.10
Shoemaking	180	2.57	1.17
Walking slowly (2.6 miles per hour)	200	2.86	1.30
Carpentry, metal working, industrial painting	240	3.43	1.56
"Active exercise"	290	4.14	1.88
Walking moderately fast (3.75 miles per hour)	300	4.28	1.95
Walking down stairs	364	5.20	2.36
Stoneworking	400	5.71	2.60
"Severe exercise"	450	6.43	2.92
Sawing wood	480	6.86	3.12
Swimming	500	7.14	3.25
Running (5.3 miles per hour)	570	8.14	3.70
"Very severe exercise"	600	8.57	3.90
Walking very fast (5.3 miles per hour)	650	9.28	4.22
Walking up stairs	1100	15.8	7.18

By the use of these estimates the probable food requirement fo a person of 70 kilograms (154 pounds) may be calculated ver simply, as, for instance, in the following example:

8 hours of sleep at 65 Calories	=	520 Calories
2 hours' light exercise * at 170 Calories	=	340 Calories
8 hours carpenter † work at 240 Calories	=	1920 Calories
6 hours' sitting at rest at 100 Calories	=	600 Calories
		3380 Calories

Tigerstedt, in his *Textbook of Physiology*, gives the following estimates of food requirements for different degrees of activity as indicated by means of typical occupations, which may be useful in checking results calculated as above:

2000–2400 Calories per day suffice for a shoemaker.
2400–2700 Calories per day suffice for a weaver.
2700–3200 Calories per day suffice for a carpenter † or mason.
3200–4100 Calories per day suffice for a farm laborer.
4100–5000 Calories per day suffice for an excavator.
Over 5000 Calories per day are required by a lumberman.

Lusk gives the following summary of energy requirements of women at work at typical occupations as investigated by Becker and Hamäläinen in Finland:

A seamstress sewing with a needle required 1800 Calories.

Two seamstresses, using a sewing machine, required 1900 and 2100 Calories, respectively.

Two bookbinders required 1900 and 2100 Calories.

Two household servants, employed in such occupations as cleaning windows and floors, scouring knives, forks, and spoons, scouring copper and iron pots, required 2300 to 2900 Calories.

Two washerwomen, the same servants as the last above named, required 2600 and 3400 Calories in the fulfillment of their daily work.

* Going to and from work, for example.

† The general belief is that Americans work more intensely, and spend more calories an hour than Europeans working at the same occupations. Hence the American example properly works out to more calories a day in this country than Tigerstedt's Scandinavian example for the same occupation.

Recommended (Daily) Dietary Allowances (RDA)

On the motion of its *Food and Nutrition Board*, the (U. S.) *National Research Council* in 1948 revised its *Recommended* (Daily) *Dietary Allowances* for healthy adult maintenance to read, as regards Calories, as follows:

Man (154 lb., 70 kg.)

Sedentary	2400	Calories	a	day
Physically active	3000	"	"	"
With heavy work	4500	"	"	"

Woman (123 lb., 56 kg.)

Sedentary	2000	"	"	"
Moderately active	2400	"	"	"
Very active	3000	"	"	"

Regarding these 1948 Recommended Allowances the National Research Council explains in its Reprint and Circular Series, Number 129 (October, 1948) that it follows the principle of recommending allowances sufficiently liberal to be "suitable for maintenance of good nutritional status." The Council further explains that its quantitative recommendations "are intended to represent exactly what is implied in a literal interpretation of the words *recommended dietary allowances*." Hence, in contrast to some previously promulgated "standards," they "are rather to be understood as representing levels of nutrient intakes which the Food and Nutrition Board recommends as normally desirable goals or objectives."

"The proper calorie allowance is that which over an extended period will maintain the body weight (or rate of growth) at the level most conducive to well-being. Due account should be taken of the concept of 'ideal weight' as developed through the study of life insurance experience. Height, age, sex, and environmental and genetic factors must also be taken into consideration. Obviously single values cannot be equally accurate for different individu-

whose needs are influenced by so many circumstances. Hence it is suggested that the recommended calorie allowances be regarded as subject to modifications of plus or minus 15 to 20 per cent according to conditions." They "are therefore not applicable to all individuals but rather represent group averages."

While most nutritionists are agreed in the view that, properly understood, the Council's recommendations are well suited to their purpose, a few critics contend that they are too high, and if actually followed would increase the obesity of which we already have too much in this country.

Discussion seems now to have made it clear that many men and women have classified themselves or been classified as "moderately active" because they are diligently engaged in their professional work; whereas if the nature of the work is chiefly sedentary they should be classified as "sedentary" and not as "moderately active."

As the terms were originally intended and have continued to be used by the National Research Council, "active" refers to muscular activity, and usually that of the larger muscles. A student or teacher or other professional worker may carry heavy responsibility but is not engaged in "heavy work" nor is he during most of the time "physically active" *with his large muscles.* When the nature of the activity contemplated by the recommendation is correctly understood, 3000 Calories a day for the 70 kg. man, physically active but not doing heavy work, and 2400 Calories for the 56 kg. woman of similar activity, do not appear excessive nor conducive to obesity if optimal amounts of exercise are taken as recreation.

A large proportion of the many dietary studies which have been published during the past 2 or 3 generations have had family or other groups, rather than individuals, as their subjects. But apparently satisfactory records have been found of 36 individual cases of sedentary men who had lived for periods of at least several months on essentially constant diets and without significant change in body weight. Their average food consumption was 2466 Calories a day.

Among methods of measuring energy metabolism, undoubtedly the most accurate, though not necessarily the most convincing because of the shortness of its periods of actual measurement, is the

method of direct calorimetry with healthy men as subjects living in respiration calorimeter as described in Chapters 8 and 9. In the 2 cases of individuals studied in this way, of which we have record the energy metabolisms of sedentary men measured by rest ex periments with the Atwater-Benedict respiration calorimeter in on of its forms were found to average 2244 Calories a day. This reaffirm the earlier series cited near the end of Chapter 8.

To the reader who has in mind the findings of Chapters 8 and 9 it will at once be apparent that even the 24-hours' metabolism measured in the respiration calorimeter *rest experiments* is not th absolute minimum to which normal adult energy output can b brought. By sufficiently rigorous resort to either of two ways (or combination of them in less drastic degree), namely complete bed rest, with corresponding reduction of food below that of the "re experiment" in the respiration calorimeter, or by persistently follow ing a sufficiently *sub*maintenance diet, an average man may brin his 24-hour energy output down to about 2000 Calories. But is th to be considered *a normal baseline*?

During World War I, F. G. Benedict, W. R. Miles, Paul Roth, an H. M. Smith (1919) studied the effects of well-marked and pro longed food restriction upon volunteer students and staff member of the Springfield, Mass., training college. These subjects receive an ordinarily well balanced diet; but only in restricted amount each man reducing his food intake until he had lost about one eight of his initial body weight during a period of 3 to 10 weeks. There after he ate only enough to maintain his weight at this reduce figure. The amount of food required to do this was 2000 to 220 Calories a day or about two thirds as much as these men were b lieved to have customarily eaten before the experiment. That is, maintain their reduced body weight at seven eighths their pr experimental average required only about two thirds their pre-e perimental food Calorie intake. This difference of *a lowering body weight by one eighth and of food intake by one third* is a counted for by the lowering of the basal metabolism during fai long periods of food restriction.

To what extent was the result a true economy in use of fo

nd to what extent did it mean the forcing of energy metabolism to n undesirably lowered plane? The men remained healthy and con-inued to perform their accustomed work, from the viewpoint of vhich facts, if taken alone, it could be argued that there was here n important gain in efficiency in that the same work was accom-olished at an energy expenditure only two thirds as large. But Benedict and Roth reported that the actual output of heat during leep was reduced by only about one fourth, which (rather than the eduction of one third) might be a truer interpretation of the actual aving of energy. In the same experiments, Smith found "a marked aving in the energy requirements for walking in favor of the re-luced diet." This saving Smith estimated at 8 per cent after 20 lays and 14 per cent after 4 months. Miles, however, reported, from is neuro-muscular and psychological studies of these same men, hat in general it must be concluded that the prolonged period of reduced diet produced some decline in neuro-muscular activities. In Miles' opinion the psychological changes due to reduced diet (in the degree here described) were not such as to interfere mate-rially with a satisfactory discharge of the common duties of a student; but he agreed with other investigators that the prolonged restriction of diet was accompanied by some lowering of "animal spirits."

On the whole it would appear that these presumably typical American young men nearly, but not fully, maintained health and efficiency at food intake levels of about 2000 Calories a day, after ample time for adjustment to this lowered level.

Do Americans eat too much? Some do and some do not. The average American probably carries some excess weight (according to the findings of life insurance experience), but probably only a few pounds.

It is also a fact to be remembered that if those men who are eating more than they need are to be bettered, there are still alternative ways: one may reduce his food intake *or* increase his energy expenditure, or both, and if the latter takes the form of home vegetable-gardening the family dietary will be consequently improved.

The Life Insurance Problem of "Ideal" Weight

Life insurance companies have made extensive statistical studie of the relation between the fatness of people, at the time of insurin their lives, and their subsequent life histories, especially as to lengt of life and recorded causes of death.

Leaving aside as a medical problem the differentiation of cause of death, we consider in this section the simpler question, what in fluence has fatness (or relation of weight to height) upon the length of life attained by accepted applicants for life insurance? Up t about two generations ago it was generally assumed by life insuranc examiners and actuarial officers that body weight a little above th average for the height and age was an indication of higher health a better-than-average life insurance "risk." By about one generatio ago this view had been modified to the belief that it was true fo the younger but not for the older people; and it became the genera teaching that the best weight for a given height was that of th average normal person at the age of 30 years.

Younger people were advised to bring themselves up to thi "standard"; but older people were advised that the further increas of weight above the age of 30 years is not advantageous, and tha they do best to hold the weight down to that which corresponds t the average *for their height and for age 30.*

Now, as the result of another generation of study, the life insur ance companies favor a lesser degree of fatness; and correspondingl they now advise *in favor of the average weight at 25 years of age*

Table 24 shows the average weights, separately for women an for men, for different heights and for ages of 25 and of 30 years side by side. From these figures it will be seen that the life insuranc "ideal weight," for height and for ages of 30 years and over, is bein reduced by only about 3 per cent from the recommendations of generation ago. This is a doubtless significant downward revisio of an expert recommendation, but it is a relatively small change only one quarter as much as the body-weight reduction imposed b Benedict, Miles, Roth, and Smith (1919). Hence, if we take accou of both kinds of evidence, it appears that the best body weight f

TABLE 24. POUNDS OF WEIGHT FOR HEIGHT AS OF AGES 25 AND 30

HEIGHT	WOMEN		MEN	
	Age 25	Age 30	Age 25	Age 30
4 ft. 8 in.	109	112		
4 ft. 9 in.	111	114		
4 ft. 10 in.	113	116		
4 ft. 11 in.	115	118		
5 ft. 0 in.	117	120	122	126
5 ft. 1 in.	119	122	124	128
5 ft. 2 in.	121	124	126	130
5 ft. 3 in.	124	127	129	133
5 ft. 4 in.	128	131	133	136
5 ft. 5 in.	131	134	137	140
5 ft. 6 in.	135	138	141	144
5 ft. 7 in.	139	142	145	148
5 ft. 8 in.	143	146	149	152
5 ft. 9 in.	147	150	153	156
5 ft. 10 in.	151	154	157	161
5 ft. 11 in.	154	157	162	166
6 ft. 0 in.	158	161	167	172
6 ft. 1 in.			173	178
6 ft. 2 in.			179	184
6 ft. 3 in.			184	190
6 ft. 4 in.			189	196

those of 30 years and over is probably somewhere between 3 and 12 per cent below the present (1951) average of American men.

Food Calories and the Control of Body Weight

While the body's weight may sometimes be significantly influenced by its water balance, in the large majority of cases over-weight means over-fatness.

And over-fatness almost always means that the intake of food calories has been out of proportion to the expenditure of energy *by the person concerned.* Undoubtedly there is more tendency to over-weight in some people than in others, and undoubtedly, too, this sometimes extends, beyond the question of appetite, to endocrine and perhaps other constitutional differences. Yet the fact remains that for the individual the control of body weight is essentially a matter of a proper balance between what is ingested as food and what is oxidized in the energy metabolism. If one tends to become too fat, the remedy is to eat less, or to burn more, or both.

To increase basal metabolism by administration of thyroid, thy-

roxine, or any drug designed to increase oxidation is too dangerous for any except rare cases under strict medical control.

To increase the energy metabolism by muscular exercise is probably beneficial in a fair proportion of cases but is usually a much slower process of weight reduction than one desires, especially as exercise usually increases the appetite. It is also to be kept in mind that strenuous exercises may be dangerous to people who are overweight as well as to those who are no longer young. Both may well remember the principle, so ably expounded by Dr. Mary S. Rose, that the only form of exercise essential to the control of body weight is the exercise of the intelligence.

Intelligent observation indicates clearly that for the greater number of people the problem is more readily solved by concentrating attention upon control of food calorie intake rather than upon increase of metabolism. Almost always there is room enough for the desirable degree of reduction of calorie intake among the foods that are not important sources of protein, mineral elements, or vitamins; and it is among such foods that the cutting out or the cutting down should be done. Several of the chapters beyond contain data that may be brought to bear upon this part of the problem.

When reduction of body weight is sought through control of the diet, one should not proceed too drastically, nor should one be discouraged or confused by "short-range" fluctuations. For, not only may the body gain or lose a few pounds of water without apparent reason or effect upon health, but also the fluctuations in the body's glycogen influence its water content also, each gram of glycogen being usually accompanied by about three grams of water. Thus 400 Calories gained or spent in the form of 100 grams of glycogen may quickly change the body weight about 400 grams, while this weight gained or lost as fat would involve about 3600 Calories.

Keeping in mind therefore that sudden fluctuations of a few pounds do not call for any change of plan, and that any weight-reduction plan should aim not at spectacular results but at steady moderate progress, it is well when reducing to deduct about 500 Calories from the daily intake which would keep the body weight constant.

Energy Allowances for Pregnancy and Lactation

Although the basal metabolism does not seem to be greatly increased per unit of weight in pregnancy, yet by virtue of her increase in weight the pregnant woman has a larger total energy requirement. The effect of increased weight is likely to be offset to some extent by the decrease in activity. During lactation, when the entire nutritive requirement of the nursing infant is being met through the mother, the energy needs of the latter are greatly increased. Production of milk involves an extra energy requirement beyond the actual energy value of the milk secreted, and the food allowance should provide accordingly. Liberal feeding of the nursing mother is not only important for the conservation of her own bodily resources but may prolong the period of lactation and thus be of great value to the child as well.

The National Research Council recommends allowances of 2500 Calories per day for women in the latter half of pregnancy, and 3000 Calories for women in lactation, stating that these figures "seek a middle ground within the somewhat broad range of present professional opinion."

Total Energy Requirements of Children

The relatively large energy requirement of children as compared with that of adults is due to a higher basal metabolism, vigorous muscular activity, and stimulation from their food intake, which must be liberal to support the storage of protein and fatty or fat-like material in the body for growth.

The Recommended Allowances of the National Research Council are: Children under one year, 110 Calories per kilogram of body weight per day; children 1–3 years, 1200 Calories (total) per day; 4–6 years, 1600 Calories; 7–9 years, 2000 Calories; 10–12 years, 2500 Calories; girls 13–20 years, 2400 Calories; boys 13–15 years, 3200 Calories; 16–20 years, 3800 Calories.

The energy costs of children's activities have not been measured very extensively as yet. In studies of infants, Benedict and Talbot

found that crying could increase the energy output as much as 200 per cent above the basal metabolism for a short time although the average increase was only about 65 per cent; while Murlin and his coworkers came to the conclusion that 30 per cent is probably sufficient allowance to make for the average amount of crying by the normal infant. In another study Benedict and Talbot reported that while vigorous crying and kicking may increase heat production by 50 per cent or more, the average increase for 24 hours may not be more than 25 per cent.

Older children were studied by Sondén and Tigerstedt, who found that in school boys and girls sitting quietly in a respiration chamber the heat production was at a level 75 and 50 per cent respectively above the basal level. Bedale measured the energy cost of several activities of boys and girls in an English private school and found, in the actual minutes of the exertion, increases of 56 to 760 per cent above the basal metabolism, according to the kind of activity. Helmreich found that the exercise of lifting the legs fifteen times a minute increased the energy metabolism of the younger children 20 to 25 per cent but that in the older children with longer legs the increase amounted to as much as 80 to 100 per cent. McClintock and Paisley found that the cost of horizontal walking in 65 boys was 20 per cent greater than that of the same exercise in men. Potgieter, measuring the carbon dioxide production of 9- and 10-year-old girls standing still, at quiet play, and climbing stairs, found elevations above the basal metabolism of 31, 90, and 290 per cent respectively.

Robb measured the energy expended during quiet play by seven 3- and 4-year-old children (4 girls and 3 boys) and found it averaged 66 and 69 per cent above the basal metabolism for the girls and boys respectively.

Robertson (Bal) measured the energy expenditure of 6- to 8-year-old children (6 girls and 6 boys) when sitting quietly and found that it averaged 55 and 57 per cent above the basal metabolism for girls and boys respectively; when cycling it averaged 242 and 229 per cent above the basal metabolism for girls and boys respectively.

Taylor, working with six 9- to 11-year-old boys, found an average expenditure above the basal metabolism of 53 per cent for quiet

play and of 212 per cent for bicycle riding. In girls in the same age range Thompson found an average expenditure above the basal metabolism of 71 per cent for quiet play and of 250 per cent for bicycling. Lamb measured the energy expenditure of 12- to 15-year-old boys when sitting quietly and found that it averaged 51 per cent above the basal metabolism; when cycling it averaged 244 per cent above the basal metabolism. (See references at the end of Chapter 9.)

With children, the energy requirement includes an additional provision for growth. In studies of infants 7 to 9 months old, Rubner and Heubner found a storage of 12 per cent of the energy value of the food consumed, and Camerer found a storage of 15 per cent of the energy and 40 per cent of the protein of the diet. An allowance of 10 to 15 per cent of the calories of the basal metabolism is generally considered a safe allowance for storage except in the periods of most rapid growth.

In order to provide adequately for all contingencies and support the rapid growth which is normal at this age, it is estimated that a vigorous child will require during the greater part of the first year about 100 Calories of food per kilogram of his body weight per day. But in cases of artificial feeding, since the digestive tract must be gradually educated to handle the milk of a different species, it will often be necessary to feed much less than 100 Calories per kilogram per day at first and gradually increase the food allowance, under the advice of the physician.

From the end of the first year until growth is completed the food requirement increases, but not so rapidly as does the body weight, so that while the allowance of food becomes larger per day it becomes smaller per kilogram of body weight.

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Chapter 11

QUANTITATIVE ASPECTS OF PROTEIN NEEDS AND VALUES

The qualitative aspects of the general and nutritional chemistry of the proteins having been discussed in earlier chapters, the purpose of the present chapter is to consider the quantitative problems of the amounts of protein needed in nutrition and the relative values of different proteins, and of the protein mixtures of different foods.

We have seen that the normal digestion of typical food proteins yields something like 20 kinds of amino acids and that 8 to 10 of these are individually essential to normal nutrition: 10 or more for rapid growth, and at least 8 of these even for adult maintenance.

We have here to study how much of average food protein we need, and then the extent to which this need depends upon the amino acid make-up of the kinds of foods consumed. Thus the problem of protein requirement becomes also a group of problems, the amount of each essential amino acid needed.

The first purpose of the present chapter is, then, to consider how much protein the body needs from day to day.

Protein Metabolism in Fasting or Deficiency

As the nature and amount of food consumed have much influence upon the rate at which protein is metabolized in the body, one's first thought might be to seek something like a "basal" rate of protein metabolism in fasting. But in fasting the energy metabolism is not greatly lowered and so the body must burn as fuel considerable amounts of the substances which it contains. Hence there is a likelihood that in fasting some protein may be used simply as fuel, which would result in an over-estimate of the true protein minimum.

Moreover, actual observations have shown that the rate of protein metabolism in fasting is at first largely influenced by one's previous habit as to the daily amounts of protein consumed and metabolized, and very considerably also by the stores of glycogen and fat available in the body for its use as fuel.

For such reasons, the nitrogen output during fasting, while often supplying data of much value in other connections, does not show us anything approximating a constant base-line for the study of the needs of normal nutrition. The presentation and discussion of numerical data regarding fasting is therefore postponed until the next chapter, where it will be of interest in connection with the corresponding data for the so-called mineral elements.

As a means of approach to the normal protein requirement, we can here more advantageously study the data of experiments designed to determine how much protein must be consumed in order to keep the adult body in protein (or nitrogen) equilibrium.

As explained in Chapter 8, quantitative determinations of nitrogen intake and output may serve as measures of the amounts of protein received in the food and metabolized in the body, figures for nitrogen being multiplied by 6.25 to obtain corresponding figures for protein. This involves the two assumptions that the food contains only negligible amounts of nitrogen in forms other than protein, and that the proteins have an average nitrogen content of 16 per cent. Each of these assumptions might be criticized in detail; but the errors involved are usually quite small in studies of human nutrition.

Nitrogen Balance Experiments and the Tendency toward Equilibrium at Different Levels of Protein Intake

The determination of the nitrogen balance has already been mentioned as one factor in the study of the total food requirement by means of metabolism experiments; and it has been shown that the *balance* is found by subtracting the output from the intake. (Theoretically the elimination through the skin should be determined and included in the calculation; practically, this is usually neglected unless, on account of warm weather or vigorous exercise, the subject

perspires profusely.*) When intake exceeds output, there is a plus balance which indicates a storage of nitrogen and therefore presumably of protein in the body; a minus balance (greater output than intake) indicates a loss of body protein. When the balance is 0, or so near 0 as to be within the limits of experimental error, the body is said to be *in nitrogen (or protein) equilibrium.*

The healthy full-grown body tends to establish nitrogen equilibrium by adjusting its rate of protein metabolism to its food supply within wide limits. The time required by the body for this adjustment depends mainly upon the extent to which the diet is changed.

The following observations by Von Noorden illustrate the establishment of equilibrium after only moderate changes in the diet:

(1) A young woman weighing 58 kilograms (128 pounds) at rest in bed was given food furnishing 1860 Calories per day (Table 25). Here there was practical equilibrium after the second day. This was a case of *adjustment to a lowered protein intake,* for the food previously taken was known to have been rich in protein.

TABLE 25. EXAMPLE OF ADJUSTMENT TO DIMINISHED INTAKE

Total nitrogen of food per day	16.96 grams
Lost in digestion (average nitrogen in feces) per day	.94 gram
"Absorbed" per day (average)	16.02 grams

	NITROGEN ELIMINATED THROUGH KIDNEYS	NITROGEN BALANCE
	Grams	*Grams*
1st day	18.2	− 2.18
2nd day	17.0	− 0.98
3rd day	15.8	+ 0.22
4th day	16.0	+ 0.02
5th day	15.7	+ 0.32

(2) Another experiment was made by Von Noorden with the same person to show the time required to reach equilibrium *after increasing the intake of protein.* In this case the food furnished 2030 Calories per day and the nitrogen balance was as shown in Table 26.

* There are some indications that frequent removal of perspiration from the skin may increase its rate of passage through the skin, so that experiments intended to measure elimination through the skin may, by their very techniques, lead to exaggerated findings.

ABLE 26. EXAMPLE OF ADJUSTMENT TO INCREASED INTAKE

AY	NITROGEN IN FOOD	NITROGEN IN FECES	NITROGEN ABSORBED	NITROGEN IN URINE	NITROGEN BALANCE
	Grams	*Grams*	*Grams*	*Grams*	*Grams*
1	14.40	0.70	13.70	13.60	+0.10
2	14.40	0.70	13.70	13.80	−0.10
3	14.40	0.70	13.70	13.60	+0.10
4	20.96	0.82	20.14	16.80	+3.34
5	20.96	0.82	20.14	18.20	+1.94
6	20.96	0.82	20.14	19.50	+0.64
7	20.96	0.82	20.14	20.00	+0.14

Here, where the amount of protein fed was increased from 90 to 130 grams per day without important change in the total fuel value of the diet, the body reached equilibrium on the fourth day after the increase.

It is apparent, therefore:

(1) That the body tends to adjust its protein metabolism to its protein supply.

(2) That when the body is accustomed to a certain rate of protein metabolism, it requires an appreciable length of time to adjust itself to a materially higher or lower rate.

Hence the rate of protein metabolism on any given day will depend in part upon the rate of metabolism to which the body has been accustomed and in part upon the protein intake for the day. When the protein supply varies from day to day, the metabolism for each day is influenced by both these factors, with the net result that the elimination tends to equal the intake when averaged for a sufficiently long period, although the data for any particular day might show a distinct gain or loss.

A transitory period of loss of nitrogen from the body is apt to be due simply to the taking of less than the usual amount of protein food; but when the body loses nitrogen for many days in succession it becomes probable that the diet is insufficient, either in total calories or in protein, to enable the usual adjustment to take place.

A transitory period of storage of nitrogen in the body may occur as the result of an increase either of the protein or of the total fuel value of the food; but a persistent storage occurs, as Von Noorden has pointed out, only under the following conditions:

(1) In the growing body (or in pregnancy) where new tissue is being constructed.

(2) In cases where increased muscular exercise results in enlargement of the muscles.

(3) In cases where, owing to previous insufficient feeding or to wasting disease, the protein content of the body has been more or less diminished and consequently any surplus available is utilized to make good the loss.

Protein-Sparing Action of Carbohydrates and Fats

It has been shown that in fasting experiments the stored glycogen and fat in the body exert a "sparing" influence upon protein metabolism, the amount of protein catabolized being smaller when the supplies of glycogen and fat are more abundant. Similarly the amounts of carbohydrates and fats in the food influence the rate of protein metabolism as indicated by the nitrogen excretion. The loss of protein which occurs on an insufficient diet may be diminished or even stopped by adding carbohydrate or fat to the food; and if carbohydrate or fat be added to the diet of a man in nitrogen equilibrium, there results a decrease in nitrogen output. If the former observation stood alone, it could be interpreted as meaning simply that the body draws upon its stored protein for energy so long, and only so long, as the fuel value of the food is insufficient; but the fact that addition of carbohydrate or fat to a diet already sufficient may cause an actual storage of protein indicates that the *protein-sparing action* or *protein-protecting power* of carbohydrates and fats involves something more than merely the question whether the body "needs" to burn protein as fuel.

As this is a matter of great importance, it may be well to consider somewhat carefully (1) the nature of the experimental evidence, and (2) a theoretical explanation regarding the protein-sparing action of the carbohydrates and fats. For an account of the earlier experiments on this subject, especially those of Voit and Rubner upon dogs, the reader is referred to Lusk's *Science of Nutrition*. Only some typical experiments upon men can be described here.

Lusk (1890), experimenting upon himself, showed the susceptibility of the protein metabolism to the sudden withdrawal of carbohydrate food. In a typical experiment, withdrawal of carbohydrate increased the nitrogen excretion from 11.44 to 17.18 grams per day.

Kayser compared the efficiency of carbohydrates and fats as sparers of protein by observing the effect upon the nitrogen balance of replacing the carbohydrates of the food by such an amount of fat as would furnish the same number of calories, and then, after three days, resuming the original diet. This experiment and that of Tallquist which follows illustrate well the methods and results of investigations based mainly upon the question of nitrogen equilibrium. Kayser, who served as his own subject, was twenty-three years old, of good physique, with a small store of body fat, and weighed 67 kilograms. In the first and third periods he ate meat, rice, butter, cakes, sugar, oil, vinegar, and salad. In the second period the diet was changed so as to consist of meat, eggs, oil, vinegar, and salad, so that nearly all of the digestible carbohydrate was withdrawn and replaced by fat. The two diets had practically the same fuel value and protein content.* The results of this experiment are shown in Table 27.

TABLE 27. NITROGEN BALANCE WHEN FEEDING ISODYNAMIC QUANTITIES OF CARBOHYDRATE AND FAT (Kayser)

PERIOD	DAY	INTAKE				OUTPUT	NITROGEN BALANCE
		Total Nitrogen	Fat	Carbohydrates	Fuel Value	Total Nitrogen	
		Grams	*Grams*	*Grams*	*Calories*	*Grams*	*Grams*
I	1	21.15	71.1	338.2	2590	18.66	+ 2.49
	2	21.15	71.8	338.2	2596	20.04	+ 1.11
	3	21.15	71.8	338.2	2596	20.59	+ 0.56
	4	21.31	71.8	338.2	2600	21.31	± 0.00
II	5	21.51	221.1	000.0	2607	23.28	− 1.77
	6	21.55	217.0	000.0	2570	24.03	− 2.48
	7	21.55	215.5	000.0	2556	26.53	− 4.98
III	8	21.10	70.4	338.2	2581	21.65	− 0.55
	9	21.10	70.4	338.2	2581	19.21	+ 1.89
	10	21.10	70.4	338.2	2581	19.65	+ 1.45

It is evident from the nitrogen balance of the first period that the amount of protein in the food was here greater than necessary, but that nitrogen equilibrium was established in four days. On substituting fat for carbohydrate (calorie for calorie) there was a marked increase of nitrogen output and, what is especially noteworthy, there was no evidence

* In experiments as short as these, it does not seem probable that the vitamin values of the diets would influence the nitrogen balances.

of any tendency to regain equilibrium during this period, but on the contrary the loss of nitrogen became greater each day the carbohydrate-deficient diet was continued. Whereas, upon returning to the normal mixed diet, not only was the loss of protein stopped, but the body almost at once began replacing the protein it had lost, although the protein and energy values of the food were practically unchanged.

Tallquist compared the protein-protecting powers of isodynamic amounts (amounts having equal energy value) of carbohydrates and fats when only a part of either was replaced by the other. The subject was Tallquist himself, a man twenty-eight years old, in good health, and weighing about 80 kilograms. The experiment was performed in Rubner's laboratory, and the diet contained such an amount of total food (36 Calories per kilogram per day) as was estimated by Rubner to be just about sufficient to supply the energy requirements of the body. The experiment covered 8 days divided into two equal periods. In the first four-day period the diet was rich in carbohydrates, in the second period it was rich in fats. There was no change in the nature of the protein fed. All foods furnishing any significant amount of nitrogen were the same in the two periods of the experiment.

The food of the first period consisted of meat, milk, butter, bread, sugar, coffee, beer. That of the second period contained the same amounts of meat, milk, bread, coffee, and beer, but less sugar, more butter, and some bacon. The same amount of salt was taken in each case. The principal data of the experiment are summarized in Table 28.

TABLE 28. NITROGEN BALANCE WHEN FEEDING ISODYNAMIC QUANTITIES OF CARBOHYDRATE AND FAT (Tallquist)

PERIOD	DAY	INTAKE					OUTPUT	NITROGEN BALANCE
		Total Nitrogen	Fat	Carbohydrates	Alcohol	Fuel Value	Total Nitrogen	
		Grams	*Grams*	*Grams*	*Grams*	*Calories*	*Grams*	*Grams*
I	1	16.27	44.0	466	18.5	2867	17.11	−0.84
	2	16.27	44.0	466	18.5	2867	14.40	+1.87
	3	16.27	44.0	466	18.5	2867	14.65	+1.62
	4	16.27	44.0	466	18.5	2867	15.58	+0.69
II	5	16.08	140.0	250	19.0	2873	17.66	−1.58
	6	16.08	140.0	250	19.0	2873	17.32	−1.24
	7	16.08	140.0	250	19.0	2873	15.94	+0.14
	8	16.08	140.0	250	19.0	2873	16.22	−0.14

Here only a part of the carbohydrate, about half of that present and an amount representing about one third of the total fuel value of the diet, was replaced by fat. The change evidently had an unfavorable

influence upon the nitrogen balance but the loss of body protein was relatively small and continued only 2 days.

Atwater compared the protein-sparing action of carbohydrate and fat in experiments in which the subject, an athletic young man of 76 kilos, performed a considerable amount of work. The experiments were carried out in the respiration calorimeter and covered in all 15 experimental days upon a diet rich in carbohydrates, arranged in four periods which were alternated with four equal periods in which the diet was rich in fats. The change from carbohydrate to fat and *vice versa* involved about 2000 Calories or nearly half the fuel value of the diet. The average results per day for the entire series of experiments were as shown in Table 29.

TABLE 29. NITROGEN BALANCE ON DIETS RICH IN CARBOHYDRATE VS. FAT (Atwater)

	ON DIET RICH IN CARBOHYDRATE	ON DIET RICH IN FAT
Available Calories in food	4532	4524
Heat equivalent of work performed, Calories	558	554
Nitrogen in food, grams	17.5	17.1
Nitrogen in feces, grams	2.5	1.7
Nitrogen in urine, grams	16.6	18.1
Nitrogen balance, grams	−1.6	−2.7

Here again there is a difference in favor of the carbohydrate, but one which is so small as to be of almost no significance.

It appears that when the carbohydrate of the food is almost entirely replaced by an equal number of calories in the form of fat there is an unfavorable effect upon the nitrogen balance; but that when the replacement is such as to affect not over one half of the total calories, the difference in protein-sparing action is but slight.

Hence under ordinary dietary conditions, the higher the total energy value of the diet, the less protein it need contain. The ability of the body to use over again some of the amino acids or even the products of their intermediary metabolism may also play a part in the ability of relatively simple nitrogen compounds to replace a part of the protein in the feeding of farm animals.

In this latter phenomenon, however, there may also be at work another and perhaps more influential factor in the synthesis of pro-

tein from simpler nitrogen compounds by bacteria or other microorganisms active in the digestive tract. Several papers dealing with investigations of this point will be found among the Suggested Readings at the end of this chapter.

Moreover, both the above processes of protein "sparing" or "replacing" by simpler nitrogen compounds may be active at the same time.

Deuel (1928) lived continuously for 54 days upon a diet nearly free from protein, consisting of starch, sugar, orange juice clarified by centrifugation, lettuce, cod liver oil, and definite amounts of pure salts. This diet contained usually from 0.24 to 0.32 gram (and never more than 0.51 gram) of total nitrogen per day, and not all of this was in the form of protein. The energy value was about 1800 Calories per day, chiefly derived from carbohydrate. On this diet the urinary output of nitrogen fell rapidly from nearly 10 grams on the first day to about 4 grams per day at the end of a week, and about 2 grams per day at the end of a month. Throughout the month however, the output of creatinine nitrogen remained nearly constant beginning at 0.60 gram and ending at 0.56 gram per day. The data of Table 30 indicate the rates of urinary output of the chief forms of nitrogen as the experiment progressed.

TABLE 30. DAILY OUTPUT OF NITROGEN IN DIFFERENT FORMS ON PROLONGED LOW-PROTEIN DIET (Deuel)

	TOTAL NITROGEN	UREA NITROGEN	AMMONIA NITROGEN	CREATININE NITROGEN	URIC-ACID NITROGEN
	Grams	*Grams*	*Grams*	*Grams*	*Grams*
1st day	9.73	7.42	0.33	0.60	0.15
2nd day	8.18	5.85	0.29	0.59	0.18
3rd day	7.35	4.99	0.22	0.57	0.20
4th day	6.05	3.88	0.20	0.57	0.19
5th day	5.49	3.49	0.25	0.57	0.18
6th day	4.82	2.76	0.21	0.57	0.15
7th day	4.42	2.45	0.24	0.57	0.17
8–9 days	3.52	2.02	0.18	0.57	0.16
10–16 days	3.29	1.87	0.17	0.56	0.16
17–23 days	2.91	1.63	0.15	0.56	0.16
24–29 days	2.32	1.24	0.10	0.56	0.14
30th day	2.10	1.14	0.10	0.56	0.13

At the end of a month on this nearly protein-free diet and when the total urinary nitrogen output had become nearly constant

about 2 grams per day, thyroxine was administered and resulted in a temporary increase of the nitrogen excretion to a maximum of 6.12 grams on the 8th day of this thyroxine period. Thereafter this nitrogen output decreased in the course of four days to about 3 grams per day and remained at this low rate notwithstanding the administration of sufficient thyroxine to maintain the increased rate of energy metabolism. The increased nitrogen excreted under the influence of thyroxine appeared almost wholly as urea, creatinine excretion remaining essentially constant. This result was taken as confirming the view that even under these conditions the extra protein metabolized was "dispensable" rather than "body tissue" protein. In discussing these results, Lusk remarked that, "The protein reserves of the body are relatively enormous," and estimated that, with carbohydrate to supply sufficient energy, a man starting from an ordinary condition of nutrition could probably live for more than a year without any protein in his food (*Science of Nutrition*, 4th Ed., p. 361).

Let us now study the data which bear directly upon the problem of the normal protein requirement.

Protein Requirement of Adult Maintenance

The practical problem may be stated thus: When the total food is properly adjusted to the size and activity of the subject so that there is sufficient but not excessive fuel to meet all the energy requirements, how much protein must the daily food contain in order to keep the body in nitrogen equilibrium?

Chittenden based his estimate of the protein requirement, not only upon the nitrogen balances, but also upon the amounts of nitrogen observed to be eliminated daily through the kidneys over long periods in which the body may or may not have been in equilibrium, but in which health and efficiency were certainly maintained. The first men to serve as subjects in his investigation were Chittenden himself and some of his associates, all of whom continued their professional work and either reported no effect or felt benefited by the change to the lower-protein diet. Similar experi-

ments were then made upon a squad of soldiers, who were quartered near the laboratory during the test and given regular exercise in the gymnasium in addition to light duties about their quarters. These men showed marked improvement in physical condition during the test, probably due in part to their more regular habits of life and their gymnastic exercises. In order to eliminate this latter factor while still applying the low-protein diet to young and physically active men, the investigation was extended to cover a group of university athletes who were already well trained and in prime physical condition at the beginning of their dietary experiment. As Chittenden emphasized, these athletes not only maintained, but in many cases improved, their gymnastic records while on the low-protein diet, one of them winning an all-round gymnastic championship during the time. Chittenden stated * that his data "are seemingly harmonious in indicating that the physiological needs of the body are fully met by a metabolism of protein matter equal to an exchange of 0.10 to 0.12 gram of nitrogen per kilogram of body weight per day, provided a sufficient amount of non-nitrogenous foods is taken to meet the energy requirements of the body." This would correspond to 44 to 53 grams of protein per day for a man of average weight (70 kilograms, 154 pounds, without clothing).

Critical Compilation of Research Data to 1920

In an examination made in 1920 of the available literature upon this subject there were found 109 experiments upon adults showing no abnormality of digestion or health, in which the diet was sufficiently well adjusted to the probable requirement, and the nitrogen balance showed sufficient approach to equilibrium, to make it appear that the total output of nitrogen might be taken as an indication of the protein requirement.

These experiments were made in the course of 25 independent investigations in which 47 different individuals (39 men and 8 women) served as subjects. For purposes of comparison the daily output of total nitrogen in each experiment was calculated to pro

* *Nutrition of Man*, pages 226, 272.

tein and this to a basis of 70 kilograms of body weight. Reckoned in this way, the apparent protein requirement as indicated by the data of individual experiments ranged between the extremes of 21 and 65 grams, averaging 44.4 grams of protein per 70 kilograms of body weight per day.

Average results for men and for women were practically identical when calculated to the same basis of body weight (for women 44.6 grams, and for men 44.3 grams, per 70 kilograms).

Study of the data recorded in the original papers indicates that the differences in amounts of protein catabolized in the different experiments cannot be attributed primarily to the kind of protein consumed nor to the use of diets of fuel values widely different from the energy requirements. Apparently, the most influential factor was the extent to which the subject had become accustomed to a relatively low protein intake such as is best calculated to throw light upon the question of the actual requirement. In the desire to avoid any danger of arbitrary selection of data, the writer probably erred in the direction of including some experiments which gave misleadingly high results because of too short periods on the low-protein diets.

A more drastic editing of the data would probably yield an average result not far from 0.5 gram of protein per kilogram of body weight per day for normal adult maintenance after allowing a reasonable period for adjustment to such a low-protein diet. Very similar requirements were found by Hegsted, Tsongas, Abbott, and Stare (1946) whose data and interpretations are cited below.

Difference between Actual Requirement and Standard Maintenance Allowance of Protein

It may be well to point out here the distinction between the amounts of protein actually required, on the one hand, and, on the other hand, the amount which it may be thought best to allow in the planning of dietaries and of food production. The term *requirement* is here applied only to the former; the latter are represented by the *Recommended Daily Dietary Allowances* of the National Research Council which are: Men (70 kg.) 70 grams; and

Women (56 kg.) 60 grams, regardless of activity and occupation, except that 85 grams are recommended for women in the latter half of pregnancy, and 100 grams during lactation.

On the question of the maintenance need of normal human adults, the protein allowance of approximately one gram per kilogram of body weight per day has been amply confirmed as generously adequate by the consistent trend of investigations from the turn of the century down to date. Professor H. B. Lewis assigns it a safety margin of 50 to 100 per cent above actual normal requirement. Leitch and Duckworth * reached essentially the same result in their restudy of all available data with use of a different mode of statistical treatment which yields them an average maintenance requirement of about 50 grams per day per normal adult.

Extensive new evidence is now available from the experiments of Stare and his coworkers † who used 26 apparently healthy adults ranging in age from 19 to 50 years. These included 5 medical students, 5 home economics students, 3 graduate nurses, 9 students from the Harvard School of Public Health, and 4 research assistants. On a basal low-protein diet in which approximately 50 per cent of the protein was supplied by white bread, 12 per cent by other cereals, 30 per cent by vegetables, and 8 per cent by fruit, protein requirements were found to be between 30 and 40 grams per 70 kilograms of body weight. When the above diet was supplemented by meat or by wheat germ even less total food protein was required for healthy maintenance. Stare and his coworkers concluded that the National Research Council's recommended daily allowance of 70 grams of protein for an adult weighing 70 kilograms "is most generous and could, if necessary, be reduced to 50 grams and still provide approximately 30 per cent margin above requirement." Stare and coworkers also consider the recent work of Bricker, Mitchell and Kinsman ‡ to be consistent with their own. Thus the results of these extensive recent investigations directly upon the protein re

* Leitch, I., and J. Duckworth 1937 *Nutr. Abs. Rev.* **7**, 257–267.

† Hegsted, D. M., A. G. Tsongas, D. B. Abbott, and F. J. Stare 194 *J. Lab. Clin. Med.* **31**, 261–284.

‡ Bricker, M., H. H. Mitchell, and G. M. Kinsman 1945 *J. Nutrition* 3 269–283.

quirement of normal human adult maintenance indicate that the margin, to cover differences of individual needs and of the protein of diets, carried by the allowance of one gram of protein per kilogram of body weight per day is at least as generous as was supposed when this level was first recommended in 1941, reaffirmed in 1945, and again reaffirmed in 1948.

The higher allowances recommended for women in the latter half of pregnancy and in lactation are based upon the research findings of Macy, Hunscher, et al., of Coons, and of others.

The Recommended Allowances for children were derived from compilations of data of nitrogen balances of children fed at different levels. These data indicate that protein intakes for each kilogram with which appropriate positive balances are obtained decrease from 4 to 3.5 gm. in infancy, 3 to 2.5 in early childhood, 2 to 1.5 in late childhood and adolescence, to the adult maintenance standard of 1 gm. The amounts in the National Research Council's table represent approximately these values. The amounts actually required vary with the size of the child and with the quality of the protein. The National Research Council's Recommendations are now usually followed.

Protein Levels in the Body

In April, 1944, a Committee on Nutrition Surveys (Dr. Charles Glen King, chairman) was set up by the Food and Nutrition Board of the National Research Council. When the war ended, the Board (in the words of its chairman, Dr. Frank G. Boudreau) "refused to discharge the Committee, insisting that its work was just as important in peace as in time of war." While the Committee divided its work into seven sections and its chairman acknowledges the contributions of its twelve other members in preparing the original manuscripts for its report, these seven sectional manuscripts had "continued study and discussions by all members of the Committee" and "review by all members of the Board, before reaching its final form."

According to this Committee report as published in 1949 (Bulletin

No. 117 of the National Research Council, *Nutrition Surveys: Their Techniques and Value*, pages 50–52) the only chemical test which has been widely applied to the question of the adequacy of the protein in our population food supply has been the determination of total serum or plasma protein. While hypoproteinemia (subnormal protein content of the blood or its serum or plasma) is readily induced when the protein in the diet is severely curtailed for long periods of time (as well as in some diseases) the Committee finds that in nutrition surveys in the United States "hypoproteinemia has been an uncommon finding" and that "dietary restrictions must apparently be more severe than those encountered in this study before a relationship between serum levels and protein intake can be demonstrated."

Present evidence therefore indicates clearly that, as compared with shortages of one or another (or more than one) nutritionally essential mineral element or vitamin value, the danger of protein deficiency in the body arising from protein deficiency in the food is (at least in Western Europe and North America) rather remote.

It is often assumed, and sometimes categorically stated, that protein consumption distinctly higher than needed is also entirely harmless; but this is not true of experiments of adequate length with rats (whose protein metabolism is much like ours) which may have their lives shortened by extra liberal protein intake as was clearly shown by the experiments of Slonaker, and confirmed by work at Columbia University.

Effects of Different Levels of Protein Intake upon Normal Nutrition throughout the Life Cycle

For obvious reasons it has not yet been feasible to extend controlled experiments upon human beings throughout the whole of their normal life cycle. This, however, can be done with experimental animals; and among these the laboratory rat is especially suitable. Among other advantages, the rat is much like man in its omnivorous food habits and in most of the chemistry of its nutrition. For these and other reasons explained in their publications, Osborne

and Mendel made much use of rats in studying the values of individual proteins for the purposes of human nutrition, as briefly indicated in Chapter 5.

Slonaker (1931, 1935, 1938) fed rats throughout their natural lives on five different diets containing approximately 10, 14, 18, 22, and 26 per cent, respectively, of protein in the dry food. The general outcome of his experiments indicated that the best permanent results were obtained with protein at the 14 per cent level (supplying 12 per cent of the total calories). Analogous full-life experiments in the Columbia University laboratories have yielded results in general agreement with Slonaker's, though somewhat more uniformly favorable through the range of 14 to 25 per cent of protein. In some of these experiments the extra protein was given as lean meat, in some as a moderately purified muscle protein preparation, and in others as casein. These results undoubtedly have significance for the human problem; though it is still an open question whether the quantitative level should be regarded as carrying over directly, or whether in view of the much slower rate at which human beings run through their life cycles and the relatively lesser demands of pregnancy and lactation in human life, we should interpret the results as indicating that the optimal average protein intake for life in the case of the human being should be a lower percentage of the total calories than found best for rats. At any rate the results of recent research confirm and strengthen the generally accepted dietetic custom of allowing from 10 to 15 per cent of the total food calories for protein under ordinary conditions.

Whether the optimal percentage of protein lies nearer to 10 or to 15 then remains an open question. To answer this question may require many and well controlled experiments extending through entire life-times and successive generations of experimental animals. And the answer may well differ according to how efficiently the amino acids supplement each other in the proteins of the experimental diet.

In later chapters and other writings we shall meet again the problem of optimal protein intake when some questions of interrelations and possible imbalances present themselves in connection with our

studies of the significance of different levels of calcium and/or riboflavin as affecting the question how much protein one can use advantageously under different conditions of calcium and riboflavin intake.

Influence of the Choice of Food upon Protein Requirement: Nutritional Efficiency of the Protein-Mixtures Contained in Different Foods

When isolated proteins are fed singly, striking differences in nutritive value appear, as has been shown in Chapter 5. In view of this fact it may seem strange that in the experiments hitherto conducted to determine the protein requirement of man the kind of protein fed has not exerted a more striking influence upon the results obtained. There is, however, no real discrepancy between the two sets of findings. The experiments made for the purpose of comparing individual proteins were conducted largely upon rapidly growing young animals, in which there is an active synthesis and retention of protein, so that a deficiency in the supply of any amino acid which is required in the construction of body protein is apt to be quickly and plainly reflected in a diminution or cessation of growth. On the other hand, in experiments like those described in the preceding section, where the purpose is not so much to compare proteins as to measure the normal protein requirement, the diet is naturally made up, not of isolated proteins or even of single or unusual foods, but (ordinarily at least) of such combinations of staple foods as are believed to represent a normal diet, so that even a relatively simple ration arranged for the purposes of such an experiment would probably contain a number of different proteins among which any peculiarities of amino-acid constitution would be apt to offset each other more or less. Moreover, the amount of an essential amino acid required for the so-called repair processes of maintenance may be relatively less than is needed in the construction of wholly new tissue in growth. Osborne and Mendel suggested that in the protein metabolism of maintenance the need for a particular amino acid may be not so much for repair of tissue as to serve as the precursor

of some essential hormone. It may also be helpful to think of the protein metabolism not only in terms of building and repair, but also of maintaining the (approximate) dynamic equilibrium which exists between proteins and amino acids in the cells of the animal tissues (Ratner, Rittenberg, Keston, and Schoenheimer, 1940).

Similarly the concept of a series of dynamic near-equilibria explains the sparing of protein by the feeding of some related compounds as well as by the nutritionally-essential amino acids themselves.

Concentration of any of the amino acids into which tissue proteins tend to be hydrolyzed may be expected to help in pushing the reaction, Amino acids $\rightleftarrows$ Protein, toward the right. In other words, *any* of these amino acids may thus function in the *maintenance* of body protein, whereas for the synthesis of new protein as in *growth, all* the amino acids which enter into the structure of tissue proteins would be needed. Hence it is quite reasonable that proteins of different efficiency for growth may show much more nearly equal efficiency in the normal maintenance nutrition of adults; though it is also true that, so far as known, the proteins more efficient for growth are likewise more efficient for maintenance.

Numerous and carefully conducted experiments have now shown the possibility of healthy adult human maintenance with nitrogen equilibrium upon dietaries furnishing not over 0.5 gram of protein per kilogram of body weight per day, even when the food protein was not of the highest nutritional efficiency as shown by growth experiments.

This was true, for example, in the experiments of Rose and Cooper with potato and of Sherman with bread as the sources of protein, in both of which nitrogen equilibrium was maintained on 0.5 gram of protein per kilogram of body weight per day; and in those of Sherman and Winters in which a young woman showed similar results upon a diet in which about nine tenths of the protein was from corn (maize) meal and about one tenth from milk.

Rose and MacLeod (1925) experimented with diets in which the protein was furnished: (1) almost entirely by meat; (2) almost entirely by milk; (3) almost entirely by a mixture of bread and milk

in such proportions that the protein came in practically equal amounts from those two foods. The food furnished 0.08 gram of nitrogen per kilogram of body weight per day. Nitrogen was stored in the body in all three cases as follows: (1) 0.06 gram per day; (2) 0.55 gram; (3) 0.41 gram. Here the protein of milk and that of the bread-and-milk mixture proved measurably more efficient than the protein of meat.

Among the western European peoples generally, whether they have remained in their homelands or settled in other parts of the world—whether more especially among the English-speaking than other peoples of European origin is not clear—there is an exaggerated tradition that good nutrition needs a high proportion of "animal protein" in an individual or family dietary, or a community or national food supply.

During the First World War and its aftermath of economic depression, some British and American investigators attained a more objective point of view as evidenced in the non-technical advice of the British physiologists; who, with the researches of Chittenden (1905, 1907) and of Folin (1905) in mind, advised their public that, "If we can take care of the calories, the proteins will take care of themselves." But the old bias of a demand for high animal protein has to some extent returned.

To free ourselves from this bias we should cultivate a habit of carrying three facts more clearly and constantly in mind: (1) Dietaries whose protein is predominantly from cereals and other foods of vegetable origin need only about ten per cent of their protein as milk to make the protein and amino acid intake excellent for maintenance and good for growth. (2) While a higher percentage of well selected animal protein induces more rapid growth, it is an open question whether the highest attainable rate of growth is the most desirable. (3) Advantages believed to have been gained from enrichments of diets in their animal protein content may actually owe their improvement to increased intake of riboflavin, or possibly in part of vitamin B_{12}, or of folic acid, or both.

Nutritionists should be careful not to exaggerate the already-existing ample (if not over-) emphasis upon animal proteins, lest soci

justice or "democratic distribution" be made more difficult by increased competition for meat in the market. And for all-round good nutrition there should be some shifting of emphasis from meat and eggs to milk and its products other than butter. This may become clearer in the study of later chapters.

Hart and Steenbock secured satisfactory growth in young swine when about one third of the total protein of the food was furnished by milk and about two thirds by maize, whereas if milk alone or grain alone were the sole source of protein, the proteins of milk showed at least twice the nutritive efficiency of the grain proteins.

As in growth, so in lactation, the demand for material for the construction of new protein creates a condition in which differences of value in the protein fed may readily become more apparent than when only maintenance is involved. Hart and Humphrey found that, in meeting the protein requirements of milch cows, milk protein and the protein of linseed meal are about 50 per cent more efficient than the proteins of corn (maize) or of the wheat kernel; and Hoobler has shown that milk is an exceptionally good form of food protein for the production of human milk and the protection of the body protein of the nursing mother.

Influence of Muscular Exercise

In order to show conclusively whether muscular work of itself has any influence upon the protein metabolism of man, it would be necessary to determine the mechanical efficiency of men, then to bring them into equilibrium with an amount of food just sufficient for their needs, and finally to have them perform a measured amount of work, at the same time adding to the diet an amount of fats and carbohydrates just sufficient to furnish the extra energy required for the work performed. Such elaborate experiments have not yet been made, but we have sufficient data to show that they are not necessary for practical purposes. Many experiments have shown conclusively that increased work, when accompanied by a sufficient increase in the amounts of fats and carbohydrates fed, does not necessarily increase the metabolism of protein.

TABLE 31. MUSCULAR WORK AND PROTEIN METABOLISM (Atwater)

NATURE OF EXPERIMENT	AVERAGE METABOLISM PER DAY					
	Per Person		*Per Kilogram Body Weight*		*Per Square Meter Surface*	
	Energy *Calories*	Protein *Grams*	Energy *Calories*	Protein *Grams*	Energy *Calories*	Protein *Grams*
Rest: Food generally sufficient for equilibrium; 5 subjects, 27 experiments, covering 82 days	2310	103.8	33.5	1.51	1116	50.1
Work: 8 hours per day. Food generally not quite sufficient for equilibrium; 3 subjects, 24 experiments, covering 76 days	4556	108.1	62.9	1.49	2129	50.5

Table 31 gives data from Atwater (*Report of the Storrs, Connecticut, Agricultural Experiment Station for 1902–1903,* page 127) which show the average results of a long series of rest and work experiments with men in the respiration calorimeter.

Comparing the figures either per unit of weight or of surface, it will be seen that muscular work sufficient nearly to double the energy metabolism had no appreciable effect upon the amount of protein metabolized as measured by the nitrogen output. Considering the large amount of exceptionally accurate research represented in these figures, they seem to justify the conclusion that if muscular work has any tendency to increase the metabolism of muscle substance, such effect is normally balanced by the tendency of the muscles to grow (and therefore to store protein) when exercised.

Moreover, it seems certain that any effect which muscular work might possibly have in increasing protein metabolism would be less than its effect in increasing the energy metabolism. If, then, starting with a diet which maintains protein equilibrium at rest, the total food is increased sufficiently to provide for the muscular work, and the increase in the diet is accomplished by adding any reasonable combination of food materials, we may feel sure that these will supply plenty of protein to meet any possible increase in the protein requirement. Hence, in planning the diet of a man at hard muscular

work, any reasonable combination of foods given in sufficient abundance to meet the energy requirement will almost certainly supply an ample amount of protein.

Protein Requirement in Relation to Age and Growth

If a man at moderately active work takes a diet which furnishes 3000 Calories and 70 to 75 grams of protein, he is taking 9 to 10 per cent of his food-energy in the form of protein. Let this be compared with the normal dietary of an infant. Human milk averages about 1.6 per cent protein, 4.0 per cent fat, 7.0 per cent carbohydrate. Here about 9 per cent of the calories are taken in the form of protein, or about the same proportion as has been allowed for the full-grown active man. Furthermore, Hoobler has shown experimentally that this is as high a proportion of protein as the infant is likely to utilize with the best efficiency in growth of body tissue. During the suckling period the growth is relatively more rapid than at any other age. Mendel * gave the following figures which have been regarded as classic:

THE RELATIVE DAILY GAIN IN BODY WEIGHT OF CHILDREN

In the first month is about	1.00 per cent
At the middle of the first year	0.30
At the end of the first year	0.15
At the fifth year	0.03
Maximum in later years	
for boys	0.07
for girls	0.04

If, then, the full-grown man and the child at the time of most rapid growth each requires but 9 to 10 per cent of his calories in the form of protein, it seems probable that this proportion is also sufficient for any intermediate age.

Recommended Allowances of the National Research Council are: Children, under 1 year, 3.5 grams of protein per kgm. of body weight per day; 1–3 years, 40 grams per day; 4–6 years, 50 grams; 7–9 years, 60 grams; 10–12 years, 70 grams; Girls, 13–15 years, 80 grams; 16–20 years, 75 grams; Boys, 13–15 years, 85 grams; 16–20 years, 100

* *Childhood and Growth*, page 18.

grams; Women, 60 grams; Men, 70 grams, regardless of the degree of muscular activity.

Discussion. Usually, a well-planned dietary for a child will show a somewhat more than average proportion of its calories in the form of protein because after weaning the main feature of the child's diet should be cow's milk, which furnishes about 19 per cent of its calories in the form of protein. A child fed mainly upon cow's milk and taking enough food amply to cover his energy requirement will therefore receive a safe surplus of protein in highly efficient form. With an optimal amount of milk in the dietary of the growing child, the other foods may be selected with primary reference to other qualities than their protein content; without a liberal use of milk the proper feeding of a growing child becomes a problem, perhaps more with respect to mineral and vitamin than to protein needs.

In general, elderly people show a somewhat diminished protein requirement and likewise a diminished power of dealing with excess. Surplus protein taken in the food is not so rapidly absorbed and catabolized, and, while there appears to be no essential difference in the form in which the nitrogen is finally excreted, the susceptibility to excessive putrefaction of protein appears to be increased. It would seem that in the dietary of the aged the protein should be reduced to at least as great an extent as are the calories, though dietary advice does not always recognize this.

Influence of Diet upon Concentration of Proteins in the Blood

In their above-mentioned study of protein requirements in human nutrition, Hegsted, Tsongas, Abbott, and Stare (1946) found that total protein, plasma albumin, and plasma globulin tended to decrease when low-protein all-vegetable diets were fed at a level low enough to produce negative nitrogen balance. Thus the level of protein intake does have *some* effect on the level of one or more of the proteins of the blood. But it does not show what blood level is optimal. This would probably require very much longer experiments, perhaps extending through whole life-times or successive genera-

tions, or both, using experimental animals of species suited to the problem.

Possibility of Protein Deficiency Conditions Not Attributable to Fault of the Food Protein

Sometimes a pathological instead of physiological functioning of the body may result in a protein-poor condition of the blood serum, although the food supplies ample protein to meet all normal needs. The paper by Weech listed below deals in part with such a condition. Although dietary measures may alleviate such conditions, nevertheless the problem is a medical one and therefore lies beyond the scope of this book. From the standpoint of the practice of medicine, the recognition of such conditions may give rise to the view that protein is deserving of renewed attention; but this does not call for any increase in our estimate of the protein needs of *normal* nutrition. Rather it calls for *discrimination* between the responsibilities of everyday food planning for normal nutrition, and the management of a medical problem in deranged metabolism.

Recommended Allowances of Individual Amino Acids

Rose's recommended daily intakes of individual amino acids for normal human adult maintenance are (1951–52) as follows: isoleucine, 1.4 gm.; leucine, 2.2 gm.; lysine, 1.6 gm.; methionine, 2.2 gm.; phenylalanine, 2.2 gm.; threonine, 1.0 gm.; tryptophane, 0.5 gm.; valine, 1.6 gm.

In each case the intake thus recommended by Rose is twice the amount which appeared to meet the minimum requirement in the average of experiments with from 8 to 31 men.

In releasing these findings, Rose urged that both the minimal and the recommended intakes be regarded as "strictly tentative."

It will be noted that these recommendations leave open the question to what extent methionine can be "spared" by cystine; or phenylalanine by tyrosine.

It is also of interest that even the broad distinction between essen-

tial and nonessential amino acids will need to be held more flexibly in the future than in the past; in the light of current research upon mutability of amino acids in experiments with tagged atoms.

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Chapter 12

MINERAL ELEMENTS IN FOODS AND NUTRITION

The Elementary Composition of the Body

From various estimates by different writers, the average elementary composition of the human body may be presumed to be approximately as shown in the first half (at the reader's left) of Table 32. The other half of the same table shows Clarke's estimate

TABLE 32. ELEMENTARY COMPOSITIONS OF THE BODY AND ITS ENVIRONMENT COMPARED

APPROXIMATE ELEMENTARY COMPOSITION OF THE ADULT HUMAN BODY (*Data from many sources*)		APPROXIMATE ELEMENTARY COMPOSITION OF THE EARTH'S CRUST WITH ITS OCEANS AND ATMOSPHERE (*Clarke*)	
Chemical Element	*Percentage*	*Chemical Element*	*Percentage*
Oxygen	65.	Oxygen	50.02
Carbon	18.	Silicon	25.80
Hydrogen	10.	Aluminum	7.30
Nitrogen	3.0	Iron	4.18
Calcium	1.5–2.2 [a]	Calcium	3.22
Phosphorus	0.8–1.2 [b]	Sodium	2.36
Potassium	0.35	Potassium	2.28
Sulfur	0.25	Magnesium	2.08
Sodium	0.15	Hydrogen	0.95
Chlorine	0.15	Titanium	0.43
Magnesium	0.05	Chlorine	0.20
Iron	0.004	Carbon	0.18
Manganese	0.0003	Phosphorus	0.11
Copper	0.00015	Sulfur	0.11
Iodine	0.00004	Fluorine	0.10
Cobalt	(c)	Barium	0.08
Zinc	(c)	Manganese	0.08
Others found, but of doubtful function		Nitrogen	0.03
		Others in lesser amounts	

[a] Estimates of normal calcium content vary widely.

[b] Phosphorus varies with calcium.

[c] Believed to be essential but as yet no consensus of opinion upon quantitative estimates.

of the elementary composition of the environment in and from which we have evolved: the earth's crust with its oceans and atmosphere. Clarke (1920) based his estimate on elaborate studies of the composition of as much of the earth's solid crust as is within ten miles of sea-level, together with the oceans and other waters of the earth's surface and the air of its weighable atmosphere. Any element which Clarke estimates as constituting less than 0.03 per cent of this total is here omitted for the sake of brevity. Of the elements reported as found in the body, we cannot always be sure which are essential to its structure or functioning and which are of accidental occurrence. All of those for which figures are given in Table 32, and also cobalt and zinc are now regarded as essential; while opinion still differs as to whether a number of other elements are nutritionally essential.

The prominence of *oxygen* in the elementary composition of our bodies is, of course, largely due to the fact that about two thirds of the body weight is water; but also oxygen is a constituent of nearly all of the compounds in the body.

Its prominence as a constituent both of water and of the organic body-stuffs gives *hydrogen* a high place in the quantitatively arranged list of the body's essential elements, and accounts for its constituting a higher percentage of the body than of its environment.

Of *carbon* and also of *nitrogen* the body contains about 100-fold higher concentration than does its environment. This is chiefly due to the fact that the body tissues are so largely composed of carbon and nitrogen compounds.

Compounds of carbon, nitrogen, hydrogen, and oxygen have been studied in part both as foodstuffs and body constituents in the preceding chapters, and will also be studied further in the chapters on vitamins.

Such other chemical elements as are normally essential to the structure or function of the body are commonly called the mineral elements of foods and nutrition, or the inorganic foodstuffs, or the ash constituents; and their transformation and "exchange" in the body collectively may be called the mineral metabolism. None of these terms is entirely satisfactory. Some of the elements which re-

main in the ash when a food is burned may have existed largely as constituents of organic compounds in the food itself. With the facts clearly understood, any of the current designations for these elements may be used.*

General Functions of Mineral Elements in Nutrition

The elements concerned in the mineral metabolism may exist in the body and take part in its functions in at least three kinds of ways:

(1) As constituents of the bones and teeth, giving rigidity and relative permanence to the skeletal tissues.

(2) As essential elements of the organic compounds which are the chief solid constituents of the soft tissues (muscles, blood cells, etc.).

(3) As soluble salts (electrolytes) held in solution in the fluids of the body, giving these fluids their characteristic influence upon the elasticity and irritability of muscle and nerve, supplying the material for the acidity or alkalinity of the digestive juices and other secretions, and yet maintaining the approximate neutrality of the body's fluids as well as their osmotic pressure and solvent power.

A man under average conditions of diet, activity, and health usually excretes daily from 20 to 30 grams of mineral salts, consisting mainly of chlorides, sulfates, and phosphates of sodium, potassium, magnesium, and calcium (as well as variable small amounts of ammonium salts from the protein metabolism).

In a fast of 31 days recorded by Benedict (1915) the elimination of several elements was determined quantitatively from day to day or in periods of two to three days with the results shown in Table 33.

It will be noted that each of the elements seems to run its own course except that (1) the sulfur tends to remain in a more or less parallel relation to the nitrogen (both being essential in protein metabolism); and (2) the output of sodium tends to run parallel with that of chlorine, since these two elements are involved mainly as common salt and its ions.

* It is, however, hardly consistent with the supposedly established usages of chemistry and mineralogy to refer to the *mineral elements* as "minerals" when these elements may be and often are present in part as organic compounds.

TABLE 33. URINARY EXCRETION OF DIFFERENT ELEMENTS DURING A 31–DAY FAST (Benedict)

DAY	NITROGEN	CHLORINE	PHOSPHORUS	SULFUR	CALCIUM	MAGNESIUM	POTASSIUM	SODIUM
	Grams	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
1	7.10	3.77	0.73	0.46	0.217	0.046	1.630	2.070
2	8.40	1.02	1.08	0.61	.243	.106	1.368	.926
3	11.34	0.79	1.10	0.68	.243	.106	1.368	.926
4	11.87	0.59	1.27	0.67	.243	.106	1.368	.926
5	10.41	0.41	1.15	0.65	.274	.098	1.445	.276
6	10.18	0.40	1.02	0.65	.274	.098	1.445	.276
7	9.79	0.55	0.80	0.62	.253	.070	.883	.154
8	10.27	0.32	0.80	0.64	.253	.070	.883	.154
9	10.74	0.31	0.93	0.66	.253	.070	.883	.154
10	10.05	0.28	0.86	0.61	.220	.072	1.006	.100
11	10.25	0.36	0.85	0.62	.220	.072	1.006	.100
12	10.13	0.31	0.74	0.62	.216	.065	—	—
13	10.35	0.32	0.85	0.62	.216	.065	—	—
14	10.43	0.26	0.81	0.60	.236	.071	.814	.109
15	8.46	0.16	0.64	0.50	.236	.071	.814	.109
16	9.58	0.14	0.89	0.59	.214	.078	—	—
17	8.81	0.12	0.87	0.53	.214	.078	—	—
18	8.27	0.15	0.81	0.54	.251	.059	.676	.051
19	8.37	0.16	0.77	0.55	.251	.059	.676	.051
20	7.69	0.15	0.64	0.51	.237	.053	.644	.066
21	7.93	0.18	0.70	0.51	.237	.053	.644	.066
22	7.75	0.21	0.69	0.50	.179	.050	.643	.083
23	7.31	0.18	0.71	0.51	.179	.050	.643	.083
24	8.15	0.10	0.68	0.49	.167	.056	.787	.065
25	7.81	0.18	0.67	0.49	.167	.056	.787	.065
26	7.88	0.16	0.65	0.54	.153	.051	.656	.055
27	8.07	0.16	0.62	0.52	.153	.051	.656	.055
28	7.62	0.14	0.59	0.53	.131	.047	.585	.036
29	7.54	0.12	0.64	0.52	.131	.047	.585	.036
30	7.83	0.14	0.61	0.52	.138	.052	.606	.053
31	6.94	0.13	0.58	0.49	.138	.052	.606	.053

In order to support permanently normal nutrition, the intake of each essential element must of course be sufficient to cover the output; and, in the case of the growing body, to provide an additional amount for daily storage as a constituent of the newly formed body substances. Instead of the former assumption, that in the practical feeding of people and of farm animals all this can safely be left to chance, there is now great interest in mineral metabolism on both theoretical and practical grounds. Not only do the different food materials differ widely in the absolute and relative abundance of

the different elements, but the same is also true of the total food intake of different groups of people. Studies of 150 freely chosen American dietaries, each covering the food of a group of people for a week or more, revealed the range shown in Table 34.

TABLE 34. MINERAL ELEMENTS IN 150 AMERICAN DIETARIES

ELEMENTS	PER MAN PER DAY			PER 3000 CALORIES		
	Minimum	Maximum	Average	Minimum	Maximum	Average
	Grams	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
Calcium	0.24	1.87	0.73	0.35	1.47	0.73
Magnesium	0.14	0.67	0.34	0.17	0.53	0.34
Potassium	1.43	6.54	3.39	1.63	5.27	3.40
Sodium [a]	0.19	4.61	1.94	0.22	4.83	1.95
Phosphorus	0.60	2.79	1.58	0.72	2.30	1.59
Chlorine [a]	0.88	5.83	2.83	0.83	7.26	2.88
Sulfur	0.51	2.82	1.28	0.80	2.35	1.30
Iron	0.0080	0.0307	0.0173	0.0090	0.0234	0.0174

[a] Since these dietary records did not show the quantities of table salt used, the figures for sodium and chlorine in the table cover only the amounts in the food as purchased and are very greatly below the actual intake of these elements.

It will be seen that the intake of any given element may be widely different in the different dietaries, even though each represents the daily average for at least a week. To some extent this is due to the variable amounts of total food consumed, but even when the data are reduced to a uniform basis of 3000 Calories the differences are still large. When we find, as here, that a day's food, even averaged over a period of a week or more, may contain anywhere from 0.24 to 1.87 grams of calcium; 0.14 to 0.67 gram of magnesium; 1.43 to 6.54 grams of potassium, it becomes probable that not all of these can have been optimal intakes, and that the mineral elements of food and their functions in nutrition are subjects calling for careful study.

The purpose of this chapter is to sketch the mineral metabolism in as concise and connected a manner as practicable, after which the more detailed and quantitative study of the elements which assume special prominence in the practical problems of nutrition will be taken up in the chapters which follow.

Metabolism of Chlorides—Use of Common Salt

Practically all the chlorine involved in metabolism enters, exists in, and leaves the body in the form of chloride ions. The amount of sodium chloride which is ordinarily added to food as a condiment is so large that the amounts of sodium and chlorine present in the various foods in their natural condition become usually of little practical consequence. But when there is profuse perspiration the water which leaves the body through the skin carries with it so much sodium chloride that it may become advantageous to replace this loss either by taking a little salt with the drinking water or by adding a little extra salt to the food.

Among animals, the herbivora require salt while the carnivora do not, the latter obtaining sufficient salt for their needs from the flesh and more especially from the blood, of their prey.

Sodium salts occur abundantly in the blood and other fluids of the animal body and in much lower concentration in the tissues. Potassium salts, on the other hand, occur to a greater extent in the soft solid tissues—in the corpuscles of the blood, the protoplasm of the muscles and other organs, and also in the highly specialized fluids which some of the glandular organs secrete, notably in milk. A conspicuous function of the salts in the tissues is the maintenance of the normal osmotic pressure, but solutions of different salts of equal osmotic pressure are for other reasons not interchangeable and it is not possible to replace successfully the potassium in the cell by an equivalent amount of sodium.

Attention is frequently called to the fact that sodium chloride is the only salt which we seem to crave in greater quantities than occur naturally in our food, and that we share this appetite with the herbivorous animals. Bunge held that this is because a high intake of potassium (as furnished by most vegetable foods) tends to increase sodium elimination. Bunge tested this theory upon his own person by taking potassium phosphate and citrate, which was found to increase the elimination of sodium chloride; and concluded that while one might live without the addition of salt to the food even on a diet largely vegetarian, yet without salt we should have a stron

disinclination to eat much of the vegetables rich in potassium, such as potatoes. While Bunge's explanations may not be entirely adequate in detail, there seems to be little doubt as to the correctness of his main deductions. They are, in general, confirmed by the more recent work of Miller (1923, 1926).

When no chloride is taken, the rate of chloride excretion falls rapidly to a point where the daily loss is only a small fraction of the amount ordinarily consumed and excreted. Thus in an experiment by Goodall and Joslin in which a healthy man was placed upon a diet adequate in protein and energy value but practically free from salt, the excretion of chlorine on each of 13 successive days was respectively: 4.60, 2.52, 1.88, 0.87, 0.69, 0.48, 0.46, 0.40, 0.26, 0.22, 0.22, 0.17, 0.17 grams.

Cetti in ten days of fasting excreted altogether 13.13 grams, and Belli in ten days on a diet poor in salt lost 11.8 grams of sodium chloride. In Benedict's study of prolonged fasting his subject lost 3.44 grams of chlorine (equivalent to 13.93 grams of sodium chloride) during the first ten days, 2.13 grams of chlorine during the second ten days, and 1.57 grams of chlorine during the third ten days of the fast. (See Table 34 above.) Since the body is supposed to contain about 100 grams of sodium chloride, it will be seen that even when there was complete deprivation of salt for as long as thirty days, the total loss did not exceed one fifth of the amount estimated as usually present in the body.

Sodium, Potassium, Calcium, Magnesium

The distribution of sodium and potassium in the body and some of their mutual relations in metabolism have been referred to in the section on the chlorides. The distribution and functions of calcium have been studied longer in somewhat greater detail than those of magnesium. It is estimated that over 99 per cent of the calcium in the body belongs to the bones, the remainder occurring as an essential constituent of the soft tissues and body fluids. Of the magnesium in the body about 71 per cent is contained in the bones. The muscles contain considerably more magnesium than calcium;

the blood contains more calcium than magnesium; and in the blood most of the calcium is in the plasma, while of magnesium the blood cells contain more than plasma.

That the calcium ion is necessary to the coagulation of the blood has long been known and frequently cited as an example of the great importance of calcium salts to the animal economy.

Calcium also functions importantly in the maintenance of the right degree of firmness in protoplasm and the soft tissues generally (L. V. Heilbrunn, *The Scientific American,* June, 1951, pages 60–63).

Striking also is the function of these salts in regulating the action of heart muscle. It is well known that heart muscle may be kept beating normally for hours after removal from the body when supplied, under proper conditions, with an artificial circulation of blood or lymph or a water solution of blood ash. Howell, Loeb, and others have studied the parts played by various salts. The sodium salts take the chief part in the maintenance of normal osmotic pressure and have also a specific influence. Contractility and irritability disappear if they are absent, but when present alone they produce relaxation of the muscle tissue. Calcium salts, also, although occurring in blood in very much smaller quantity, are absolutely necessary to the normal action of the heart muscle; while if present in concentrations above normal, they cause a condition of tonic contraction ("calcium rigor"). There is therefore a balance which must be maintained between calcium on the one hand and sodium (and potassium) on the other. Thus it is found that the alternate contractions and relaxations which constitute the normal beating of the heart are dependent in part upon the presence of a sufficient but not excessive concentration of calcium salts, and in part upon the quantitative relationship of calcium to sodium and potassium in the fluid which bathes the heart muscle. Other active tissues of the body doubtless have analogous requirements as to inorganic salts.

Regarding the adequacy of the ordinary intake to meet the specific requirements for sodium, potassium, calcium, and magnesium it would seem that only in the case of calcium (among these) is it ordinarily necessary to take thought in the selection of food ma

ials or in the planning of human dietaries. The amount of sodium chloride usually added to food is more than sufficient to meet the sodium requirement of the body. Potassium and magnesium are relatively abundant in a wide variety of plant and animal foods, so that an ordinary mixed diet, unless it consist too largely of highly refined food materials, will usually furnish a safe surplus of these elements. Dietaries entirely adequate in energy value and protein content may, however, contain too little calcium. Calcium requirement is therefore a question of much practical importance in human nutrition, and requires quantitative study, which will be taken up in Chapter 14.

On the other hand, some physicians suspect that too high intakes of sodium, from excessive additions of table salt to food, may aggravate a tendency to too high blood pressure. "Sodium-free" diets are sometimes recommended for this reason; but Allen, from extended clinical study of the question, suggests that simple avoidance of foods containing *added* salt probably makes the sodium intake low enough for clinical needs.

Phosphorus, Silicon, Fluorine, Iodine

Mellor quoted the saying that "if the biographies of the elements could be written, that of *phosphorus* would be the most interesting of all," and Peters and Van Slyke long ago cited fourteen ways in which compounds of phosphorus may function in the body. Without attempting an enumeration of the nutritional functions of phosphorus, attention may be called to the fact that the phosphorus compounds concerned in the processes of nutrition include derivatives of all the main groups of organic nutrients, as well as mineral phosphates both readily soluble and relatively insoluble.

Phosphorus may be a limiting or determining factor in human nutrition, in that of farm animals, or in the nutritional and evolutionary processes of nature. Both in the sea and on the land, the available supply of phosphorus may determine the growth, composition, and multiplication of plants and through them of animals also. The quantitative aspects as affecting human nutrition are discussed in Chapter 14.

Bunge and Abderhalden have emphasized the parallelism betwee the phosphorus and calcium content of milk and the rate of growt of the young, in different species (Table 35).

TABLE 35. RELATION OF MINERAL ELEMENTS OF MILK TO RAT OF GROWTH (Bunge and Abderhalden)

SPECIES	NO. OF DAYS REQUIRED TO DOUBLE THE BIRTH WEIGHT	PERCENTAGE COMPOSITION OF MILK (Partia			
		Protein	Ash	Calcium	Phosphorus
Human	180	1.6	0.2	0.02	0.02
Horse	60	2.0	0.4	0.09	0.06
Cow	47	3.5	0.7	0.12	0.09
Goat	22	3.7	0.78	0.14	0.18
Sheep	15	4.9	0.84	0.18	0.11
Swine	14	5.2	0.80	0.18	0.14
Dog	9	7.4	1.33	0.32	0.22
Rabbit	6	14.4	2.50	0.65	0.43

The bones and teeth, which contain over 99 per cent of the ca cium of the body, are estimated to contain about 90 per cent of i phosphorus. The calcium and phosphorus of the bones and tee are now said to be present essentially as crystal structures corr sponding to those in phosphate rocks, and to be capable of repr sentation by a mineral formula such as: $CaX_2 \cdot n[Ca_3(PO_4)_2]$; which X_2 is largely represented by CO_3 but may be in part also F_2, $(OH)_2$, and possibly others; and n is usually between 2 and The chemistry of the bone salts is fully discussed by Logan (1940 and reviewed in *Nutrition Reviews* for August, 1949.

Our skeletal structures also resemble the phosphate rocks containing small and variable proportions of *silicon* and of *fluori*

Silicon, while it has been regarded by some as a regular consti ent of the enamel of the teeth, we can probably dismiss along w aluminum from any serious consideration *pro* or *con* in our prese study. Our bodies have evolved in, and we live in, an environme so rich in aluminum and silicon compounds that it seems impr able that we should be injured by the small amounts of our us incidental intake; or that we should need more than we regula get incidentally.

Fluorine presents a different sort of problem. It is very uneve

distributed in nature, and the natural waters of some localities contain enough to have deleterious effects.

Mottled enamel, a disease of human teeth, has been found by Smith, Lantz, and Smith (1931) to be due to fluoride naturally present in the drinking water of the afflicted communities. The significance of fluoride in much lesser concentrations is still a problem of active research. (See Sherman and Lanford's *Essentials of Nutrition*, 3rd Ed., Ch. 18.)

The minute amount of *iodine* (iodide) in drinking water, however, is of great importance as a chief (often, if not usually, the chief) source of iodine for the manufacture in the thyroid gland of the metabolically essential substance thyroxine. Because of its importance, a separate chapter will here be given to the study of the nutritional role of iodine and its relation to the prevention of goiter (Chapter 16).

Iron, Copper,* Zinc, Manganese,* Cobalt

Iron, like phosphorus, is a constituent of the chromatin substances which are often referred to as concerned with the most vital activities of the cells, and recently it appears that a small amount of iron has an essential relation to the oxidation-reduction functioning of cytochrome; but much the largest part of the iron in the body exists as hemoglobin in the blood. In round numbers, the blood constitutes only about 7 per cent of the weight of the body, but contains about 60 per cent of its total iron. The hemoglobins in different species of vertebrates differ but little; all are iron-containing proteins constituting a considerable part of the red blood corpuscles and functioning as oxygen carriers in respiration.

Copper, long known to be a constituent of certain lower animals, has also been found to function in minute amounts in the nutrition of the higher animals, as is explained in Chapter 15, in which the functions of iron and copper in nutrition will be taken up more

* In the Seventh Edition of this book, pages 630–632, may be found a tabulation of copper and manganese values of foods which exigencies of space have made it necessary to omit from the present edition.

fully. As the functioning of copper in mammals is, so far as known, chiefly as catalyzing the formation of hemoglobin and as taking a related part in the oxidation process, its further discussion is taken up in Chapter 15.

Zinc is widely distributed in plant and animal tissues. Mitchell (P. H., 1950) considers that zinc is indispensable in human nutrition but not a problem in dietetics or consideration of the human food supply inasmuch as "human deficiencies are not known to occur."

Manganese and *cobalt* are now believed to be essential in mammalian nutrition, the latter as an element in vitamin B_{12}. Their further functions in the human body are, however, not clearly understood. It is not generally believed that either of these elements is apt to be a limiting factor in practical problems of dietetics or human food supply.

Sulfur

Plants synthesize amino acids, proteins, glutathione, and thiamine using the sulfur of sulfates that they absorb from the soil. Thioneine, the betaine of thiolhistidine, is probably formed in the animal body as are also, of course, many proteins.

Much the largest part of the sulfur of the body and its food is in the form of protein.

To a great extent, therefore, the metabolism of sulfur may be regarded as a part of the protein metabolism, and in many respects the metabolism of sulfur tends under normal conditions to run parallel with that of nitrogen. In a series of ten experiments (each of 3 to 5 days' duration) upon man, in which the food consisted of bread and milk in varying amounts and proportions, the percentage absorption from the digestive tract was nearly the same for the sulfur as for the nitrogen of the food, and the excretion of the end products ran so closely parallel that in every case in which the body stored nitrogen it also stored sulfur, and *vice versa*. Such parallelism, however, is not always found (Fay and Mendel, 1926; Lewis, 1924).

It is well known that individual proteins show relatively much greater differences in sulfur than in nitrogen content, so the ratio

f nitrogen to sulfur varies widely, as is shown by the following examples selected from the data for pure proteins compiled by Osborne (Table 36).

ABLE 36. NITROGEN AND SULFUR IN TYPICAL PROTEINS

IND OF PROTEIN	NITROGEN	SULFUR	RATIO OF NITROGEN TO SULFUR
	Per Cent	*Per Cent*	
egumin	18.04	0.385	46.9 : 1
ein	16.13	0.600	26.9 : 1
destin	18.69	0.88	21.2 : 1
liadin	17.66	1.027	17.2 : 1
eucosin	16.80	1.280	13.1 : 1
asein	15.78	0.80	19.7 : 1
yosin	16.67	1.27	13.1 : 1
erum globulin	15.85	1.11	14.3 : 1
gg albumin	15.51	1.616	9.6 : 1

Thus, while many proteins approximate the usually assumed average of 16 per cent nitrogen and 1 per cent sulfur, there are considerable deviations from this ratio in both directions.

Under ordinary conditions, however, it is the proportion of sulfur to the total protein of the food which determines the ratio of sulfur to nitrogen available for nutrition. Sulfur and protein have been determined in most staple foods, of which the data in Table 37 are representative.*

Taking these figures as typical, it would appear that in those staple foods which contribute the greater part of the protein of the diet, the ratio of protein to sulfur does not differ greatly, and that in most cases of ordinary mixed diet there would be consumed not far from 1 gram of sulfur in each 100 grams of protein. We may therefore expect that in health and on an ordinary diet the sulfur requirement will usually be covered when the protein supply is adequate.

It is chiefly the oxidation (to sulfate ion) of the sulfur taken into the body as protein which gives rise to the problem of fixed-acid elimination in acid-base balance.

* In the data here given, nitrogen and sulfur were determined in the same specimens of proteins. Average amounts of protein and sulfur in many important food materials may be found in the Appendix.

TABLE 37. SULFUR CONTENT OF FOODS IN PERCENTAGE OF THEIR PROTEIN CONTENT

FOOD MATERIAL	SULFUR IN PERCENTAGE OF TOTAL PROTEIN
Lean beef	0.95–1.00
Eggs	1.4
Milk	0.95–1.09
Wheat flour, crackers	1.15–1.29
Entire wheat	1.30
Oatmeal	1.55
Beans	0.69–1.00
Peas	0.80–0.94
Potatoes	1.07

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Chapter 13

NUTRITIONAL ASPECTS OF ACID–BASE BALANCE

Notwithstanding the large amounts of acid produced in metabolism, the reaction of the blood plasma normally remains remarkably constant and slightly alkaline, between about pH 7.33 and pH 7.51 (Earle and Cullen, 1929). In healthy individuals, forced breathing or strenuous exercise may cause a departure from these limits for short periods of time; but about pH 7.0 to pH 7.8 is the extreme range reported compatible with life.

Buffers of the Blood

One reason that tne blood can receive such large amounts of the acid products formed by the cells with so little change in its "reaction" (hydrogen ion activity, pH) is that it is highly buffered. Quantitatively the most important buffer systems of the blood are those of the proteins and their alkali salts. As has previously been pointed out, proteins are amphoteric substances capable of reacting either as acids or as bases. Since the chief proteins of the blood have isoelectric points more acid than the natural medium in which each occurs, they are presumably present at this hydrogen ion activity as buffer systems made up of the protein in its acid form (HP), with an excess of its alkali salt (B^+P^-). Van Slyke states that about one tenth of the buffer power of the blood is attributable to the plasma proteins, much the greater part of the remainder being due to hemoglobin. This is partly because of the higher concentration of the latter (human blood contains in 100 cc. about 13 to 16 gm. hemoglobin and 3.5 to 4.0 gm. plasma protein), but also because at the hydrogen ion concentration of the blood, the *buffer value* of hemoglobin is more than twice as great as that of plasma proteins (Hastings, et al., 1924).

Hastings and Eisele (1940) found the acid-base balance of the blood of a normal adult at rest "to vary (in a regular, not random manner) during a 24-hour period due to . . . (*a*) a rise in the plasma HCO_3 associated with the ingestion of food; (*b*) an increase in the CO_2 tension of the blood associated with sleep."

Phosphates in the blood form buffer mixtures made up of the acid ($H^+B^+HPO_4^=$) and its salts ($B^+B^+HPO_4^=$). A buffer system is most effective at a hydrogen ion activity approximating the dissociation constant of the buffer acid. Since the dissociation constant (pK) of monobasic phosphate at physiological salt concentration is about 6.8, the reaction of the plasma is favorable for the buffer activity of the phosphates. However, their concentration in the blood is so low that their buffer effect here is overshadowed by that of the proteins.

Elimination of Carbonic Acid

The bicarbonate-carbonic acid system has little buffer effect since in solutions of the same ionic strength as blood plasma, the maximum buffer efficiency is observed at about pH 6.1, far from the pH of the plasma. Nevertheless, the blood bicarbonate has a special significance in the maintenance of a constant hydrogen ion activity within the body, since, because its acid is volatile, the large store of base combined in this way is available for the neutralization of acids other than carbonic. On the other hand, any base which is present in excess of acids other than carbonic takes the form of bicarbonate. Because of these properties the bicarbonate of the blood has been called the *alkaline reserve*. It is considered by Peters and Van Slyke as reflecting more or less closely the reserve of available alkali present in the body as a whole.

Of the acids which, in the normal process of metabolism, are continually formed in the cells, carbon dioxide is quantitatively by far the most prominent. The means by which it is transported from the cells to the lungs to be eliminated, without marked change in the hydrogen ion activity of the blood, are of special interest. To some

extent, carbonic acid, like any other acid, is neutralized by the blood buffer systems which have been discussed. In addition, hemoglobin is rather uniquely adapted to function as a carrier of carbon dioxide as well as of oxygen. In the lungs, where the oxygen tension is high, hemoglobin combines readily with oxygen to form oxygenated hemoglobin; this in turn gives up its oxygen when it reaches the tissues, where the oxygen tension is low. Since the oxygenated form is a distinctly stronger acid than the reduced form, the change in the state of oxidation of hemoglobin which occurs at the tissues is similar to the removal of acid. Less base being required to neutralize the weaker acid (reduced hemoglobin), more is made available to react with the increased amount of carbonic acid which is being received simultaneously by the blood. On the other hand, at the lungs, where hemoglobin is reoxygenated, the effect upon the bicarbonate of the blood is similar to the addition of acid, and results in an increased formation of carbon dioxide which is excreted.

Conversely, the tendency to increased acidity resulting from the higher tension of carbon dioxide in the tissues causes an increase in the dissociation of oxygenated hemoglobin into oxygen and hemoglobin, i.e., tends to increase the liberation of oxygen in the tissues. Thus, the formation of oxygenated hemoglobin in the lungs directly increases the excretion of carbon dioxide while the increased tension of carbon dioxide in the tissues increases the liberation of oxygen from combination with hemoglobin.

Roughton and others have stressed the possibility that hemoglobin may combine chemically with carbon dioxide, the compound (or compounds) thus formed, *carbhemoglobin*, aiding significantly in the carrying of carbon dioxide in the blood.

Another constituent of the red blood cells, an enzyme (*carbonic anhydrase*) which accelerates the dehydration of carbonic acid to carbon dioxide and the reverse conversion of carbon dioxide to carbonic acid, assists in the interchange of gases both in the lungs and in the tissues.

From the discussion thus far, it appears that the cells of the blood

are richly buffered; the plasma, on the other hand, is only poorl buffered. Van Slyke and his coworkers have shown, however, tha under physiological conditions, the buffering ability of the cells shared with the plasma by an exchange of ions known as th *chloride shift*. There is also evidence that other anions to which th cell membranes are permeable, such as sulfate and phosphate, ma undergo a similar redistribution with changing concentrations c carbon dioxide, but their influence is relatively slight because c their much lower concentration.

A very important factor in the elimination of carbon dioxide an in the regulation of the acid-base balance of the body is the fac that there is a tendency for the respiratory process to hold the ten sion of carbon dioxide in the blood nearly constant. Both the dept and the frequency of breathing are supposed to be under the contro of the so-called *respiratory centers* in the brain, the activity c which is most directly influenced by the pH of the cells in the cente (Gesell, 1925). An increased production of carbon dioxide as th result of accelerated metabolism, during exercise, for example, tend to lower the pH of the blood, and consequently, of the cells in th respiratory center. This change stimulates breathing so that mor carbon dioxide is eliminated by the lungs, and the hydrogen ic activity and the carbon dioxide tension of the blood are restore toward normal. According to Henderson (1928), "it is the changin ventilation of the lungs that most directly controls the acid-bas equilibrium of the blood."

Elimination of Fixed Acids

In addition to carbonic acid, which is volatile and therefore readi eliminated from the lungs, non-volatile or fixed acids are also pr duced in metabolism and must be excreted. This involves two ma steps: the immediate reaction of the acids with the bicarbonat and buffer salts of the blood or tissues; and the subsequent elimin tion of these acids as salts by the kidneys.

The action of the bicarbonates and buffers of the blood in neutr izing these acids immediately as they are formed is indeed a m

important safeguard for the organism; but it is only a temporary measure, as the salts of the fixed acids formed in this way remain to be excreted and their removal from the blood involves the simultaneous removal of basic substances as well. In itself, this would tend to reduce the bicarbonate concentration or the alkali reserve of the blood and to result in the loss of fixed base from the body. It is here that ammonia and the buffer action of the salts of the urine play an important role.

The kidney has the ability to form ammonia, which combines with the acid to be excreted, replacing in part the fixed bases, which latter are returned to the blood in the form of bicarbonates. The amount of ammonia so formed varies directly with the amount of fixed acid to be excreted; and apparently is derived from glutamine and, to a lesser extent, from amino acids (Van Slyke, et al., 1943, Lotspeich and Pitts, 1947).

In contrast to that of the blood, the hydrogen ion activity of the urine varies rather widely, from about pH 4.8 to about pH 8.4, depending upon the proportions of acid and basic products formed in metabolism and upon the amounts and proportions of these products which are removed from the blood through other channels (particularly the lungs and the gastrointestinal tract). Strong acids such as hydrochloric and sulfuric cannot exist as such, to any appreciable extent, in solutions of the hydrogen ion activity of the urine and must therefore be excreted with their chemical equivalent of base. On the other hand, weaker acids, such as monobasic phosphate and some of the organic acids of the urine, act as effective buffers in acid urine, an increased acidity of the urine resulting in the excretion of a larger fraction of the buffer in the acid form. For example, at pH 7.4 in the blood, about 80 per cent of the total inorganic phosphate is in the dibasic form, while at pH 4.8 in extremely acid urine, about 1 per cent is in the dibasic form, the remainder being in the monobasic form. In other words, by excreting a urine of pH 4.8, the body is able to conserve about two fifths of the base which had been combined in the blood with the excreted phosphate.

Excretion of Organic Acids

It is comparatively rare under normal conditions that a freely chosen human diet gives rise to any considerable preponderance of basic products in metabolism. However, when this occurs, or when there is a temporary loss of acid as during active gastric secretion, the kidney assists in the maintenance of a constant hydrogen ion activity within the body. The urine becomes alkaline, with the result that the phosphate combines with relatively more fixed base. The excretion of ammonia practically ceases, fixed base being used instead for the neutralization of organic and inorganic acids. A large part of the excess base is eliminated as bicarbonate. The organic acids of the urine are of practically no significance *as buffers* under these conditions, since at the pH of the blood they exist almost wholly as neutral salts, and consequently can combine with little more base even at a more alkaline pH. In many cases of alkali excess, however, it has been noted that there is an *increased formation* and excretion of organic acids, these additional quantities of acid removing from the body their equivalent of fixed base. Citric acid has been studied particularly in this connection, but it is said that lactic, beta-hydroxybutyric, hippuric, and other organic acids may also occur in larger amounts when the urine is more alkaline. It has been found (Sherman, Mendel, Smith, and Toothill, 1936) that the urinary excretion of citrate for any given individual tends to parallel closely from day to day the pH of the urine, rising with an increase in alkalinity, regardless of the nature of the dietary change which caused that increase. Under usual dietary conditions, the excretion of citric acid varies from about one quarter of a gram to one gram, but when sodium bicarbonate is taken by mouth, more than two grams of citrate may be present in the urine. It appears, therefore, that citric acid, which the body can synthesize, may have some importance in conserving the fixed acid of the body when there is an excess of basic elements to be eliminated. However, it must be emphasized that the amount of base which is removed in combination with the organic acids when the urine is alkaline is only a small fraction of that which may be eliminated as bicarbonate.

Following is a list of some of the organic acids which have been found in normal human urine, with their ionization constants and approximate figures for the amounts excreted daily under usual conditions. The list is incomplete and the data in most cases only semi-quantitative because of the lack of satisfactory analytical procedures for identifying and determining specifically the individual acids.

TABLE 38. ORGANIC ACIDS OF URINE

ACID	DISSOCIATION CONSTANT K_a	NORMAL EXCRETION Mg. per Day
Citric	K_1 8.3×10^{-4} K_2 4.1×10^{-5} K_3 3.2×10^{-6}	200–1200
Uric	1.5×10^{-6}	200–1000
Hippuric	2.2×10^{-4}	100–1500
Lactic	1.3×10^{-4}	70–100
Formic	2.1×10^{-4}	10–100
Oxalic	3.8×10^{-2}	10–50
β-hydroxybutyric	3.9×10^{-5}	0–75

Influence of Diet

The normal fluctuations of fixed acid production in healthy man depend upon the diet. If the proportions of fat, carbohydrate, and protein are so unbalanced as to upset the normal ketogenic-antiketogenic relationship, the body may be flooded with organic acids arising from incomplete oxidation of fat. This is the same state that occurs in diabetic acidosis. Such a ketosis is also common in starvation and is artificially produced for therapeutic purposes by a ketogenic (high fat, low carbohydrate) diet. It is, however, presumably rare in normal individuals on self-chosen diets and is not what we are concerned with in this discussion.

Since the non-volatile end products of normal metabolism are almost entirely inorganic in nature (with the exception of urea, which is neutral in reaction), an approximate estimate of the potential acid- or base-forming properties of a food may be made from a consideration of the amounts and proportions of the inorganic elements which it contains. A fairly large number of foods were analyzed and their balance of acid-forming and base-forming ele-

ments computed by Sherman and Gettler (1912). For this calcula tion, the amounts of chlorine, phosphorus, and sulfur present wer expressed as the equivalent number of cubic centimeters of norma acids; the amounts of calcium, magnesium, potassium, and sodiu present were expressed as the equivalent number of cubic centi meters of normal alkali; and the predominance of acid-forming o base-forming potential was obtained by difference. Typical result are shown in Tables 39 and 40.

TABLE 39. EXAMPLES OF FOODS IN WHICH ACID-FORMING ELEMENTS PREDOMINATE

FOOD (Edible Portion)	APPROXIMATE POTENTIAL ACIDITY (cc. Normal Acid)	
	Per 100 Grams	*Per 100 Calories*
Beef, clear lean round steak	12	10
	11	7
Eggs	11	7
Oatmeal	12	3
Oysters	15	30
Rice	9	2
Wheat, entire	12	3
Wheat flour	9	2
White bread, made with water	6	2

It should be emphasized that this method of calculation wa adopted for the sake of simplicity and that its simplifying assump tions are professedly only approximately correct. For instance, phos phorus is calculated as a dibasic acid, although we have pointed out above that at the pH of the blood it combines with about 1.8 equivalents of base. The assumption that the sulfur is completely oxidized and is neutralized entirely by inorganic base is also only an approximation; and the organic acids and carbon dioxide are entirely omitted from these calculations of the balance of acid-form ing and base-forming elements of the food.

Notwithstanding these limitations, the results calculated from the mineral analyses of foods have in practice shown a general relation ship to the effects of the foods on the acid-base balance of the body as indicated by the urinary acidity and ammonia, except in those cases in which the food contains a considerable amount of some

organic acid which is not oxidized in the body. The foods of which this is known to be true are omitted from the accompanying tables. The mineral contents of commercial fats, sugars, and starches are too low for these to have any significant effect upon the acid-base balance, so they also are omitted from these tables.

TABLE 40. EXAMPLES OF FOODS IN WHICH BASE-FORMING ELEMENTS PREDOMINATE

FOOD (Edible Portion)	APPROXIMATE POTENTIAL RESERVE ALKALINITY (cc. Normal Alkali)	
	Per 100 Grams	*Per 100 Calories*
Apples	3	6
Bananas	8	8
Beans, dried	10	3
string, fresh	5	13
Beet, fresh	10	25
Cantaloupe	7	18
Carrots	14	30
Citron	9	3
Dates	9	3
Lemon or juice	4	10
Onions	1	2
Orange or juice	5	10
Pears, fresh	4	5
Potatoes	9	10
Radishes	5	23
Rutabagas	8	29
Sweetpotatoes	6	5
Tomatoes	5	24
Turnips	11	33
Watermelon	4	12

In experiments on man, Sherman and Gettler (1912) studied the effect of replacing the potato of a mixed diet by rice. It was found that 33 per cent of the calculated increase of fixed acid from metabolism was accounted for by an increase in the urinary ammonia, and about 40 per cent by the increased titratable acidity of the urine. The remainder of the excess fixed acid may have been eliminated, in part at least, through the skin or in the feces, or may have been neutralized by the buffers of the blood with a corresponding increase in the carbon dioxide output and decrease in the reserve alkalinity of the body. In this experiment, the intake and output of phosphorus were approximately the same on the two diets. The increased acidity

of the urine, therefore, implied an increased ratio of primary to s
ondary phosphate in the urine but not necessarily an increase
the amount of fixed base leaving the body. In the neutralization
sulfuric acid by means of phosphate, each molecule of the a
(representing one atom of sulfur oxidized to sulfate in protein meta
olism) changes two molecules of secondary into primary phospha
Whether or not this results in increased excretion of phosphate a
therefore of fixed base (or only, as in the experiment just cited,
an altered ratio of primary and secondary phosphates in the urin
apparently depends upon a complex combination of conditions
yet fully understood.

Many fruits and fruit juices contain considerable amounts
organic acids, of which citric acid is perhaps the most familiar. M
of these are di- or tribasic acids and exist in the fruits partly
potassium acid salts and partly as free acids. As eaten, these fru
have an acid reaction; whether they are potentially acid or pote
tially basic in metabolism depends upon the extent to which t
organic acid radicles are oxidized in the body, with the formation
potassium bicarbonate. Thus tomatoes, oranges, pears, peach
apricots, and pineapples tend to diminish the acidity of the urin
while, on the other hand, cranberries, plums, and prunes usually i
crease urinary acidity. The explanation for the behavior of th
latter group has been found in the presence in these fruits of rel
tively large quantities of quinic acid, which, instead of being o
dized completely in the body, is converted into hippuric acid whi
appears in the urine. It has been found that when human bein
take by mouth as much as forty grams of citric acid (more tha
it seems likely that they would ingest per day in their food), le
than 5 per cent of the acid appears in the urine (Sherman, Mend
Smith, and Toothill, 1936a). Less is known of the fate of malic ac
in the human organism, although cats, rabbits, and dogs can destr
it to a large extent. Human tissues apparently have almost no abili
to oxidize tartaric acid. When tartrates or grape juice are taken b
mouth, however, only a small fraction of the acid reappears in th
excreta, the greater part being destroyed by the microorganisms
the gastrointestinal tract. The observation that the ingestion of gra

products may, under favorable conditions, decrease the urinary acidity has been explained by the absorption of potassium bicarbonate which is a probable end product of bacterial action on the potassium acid tartrate of the grape.

Oxalates occur in small amounts in a great many fruits and vegetables, and in very considerable quantities in certain plants, especially of the family Chenopodiaceae, including beet greens, chard, spinach, and sorrel. Little is known concerning the ability of the body to metabolize oxalate. Moreover, the insolubility of calcium oxalate throughout the digestive tract makes it questionable how much of the oxalic acid of the food is actually absorbed; and, conversely, oxalate in the food may greatly reduce the absorption of the food calcium (Fincke and Sherman, 1935). Oxalic acid in small amounts is a normal constituent of the blood and urine, and probably, like citric acid, it is formed in metabolism. These considerations make it impractical to discuss oxalate-rich foods from the point of view of their effect on the acid-base economy of the body.

The question, whether or not there is merit under ordinary conditions in so choosing the food that the acid-forming elements thus introduced into the body shall be balanced by equivalent amounts of base-forming elements, must, in the opinion of the writer, be regarded as still open at the present time (1951–52). Much the greater part of the research which bears upon the question has been directed toward the elaboration of the theory of the maintenance of acid-base balance *in the blood*. So brilliant have been the results that there has been a tendency to overlook the limitations. Relatively little attention has yet been given to the important findings of Rous (1925) that all or most of the body tissues are somewhat less alkaline than the blood plasma, while the organs in which the nutritional processes are particularly active are, or may be, frankly acid (around pH 5.6). Thus some of the most important parts of the body get only partially the benefit of the buffering capacity of the blood; and so may be more influenced by an excess of acid-forming elements in the food than the studies of the blood would seem to suggest.

The working-out of the problems of the buffers and the carbonic acid carrying capacity of the blood is an outstandingly brilliant

triumph of physico-chemical research. This knowledge of the blood however, does not solve the problems of acid-base balance in all th separate organs of the body, nor permit one to say with confidenc whether the balance of acid-forming and base-forming elements i food is or is not of practical significance in human nutrition.

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Chapter 14

QUANTITATIVE ASPECTS OF CALCIUM AND PHOSPHORUS NEEDS AND VALUES

The qualitative functioning of calcium and phosphorus in huma nutrition having been sketched in Chapter 12, the present chapt will deal chiefly with the quantitative questions: how much needed; how much is best; and how do different foods compare sources of calcium (and phosphorus) in normal human nutrition?

There is not now much disposition to discriminate between organ and inorganic forms of calcium or phosphorus in foods, in meetin the nutritional needs of the body. Mainly, this quantitative stud deals with the body's intake and output of the total amounts of thes elements, without much subdivision as to forms of chemical com bination; and such will be our plan here. The first and main pa of the chapter is devoted to the body's intake and output of calciu (and more briefly, of phosphorus) as a whole. This, then, can serv as a general baseline and framework of any more specific studie of the values of different foods as sources of calcium in nutrition.

In general, the well nourished body so uses its supplies as to kee the calcium content (or, more strictly, the concentration of calciu ions) of the *blood* very nearly constant. This relative constancy o the blood calcium is largely due to the *bone trabeculae*. These ar crystals of calcium compound which grow from the inner surface o the ends of the bones, projecting from all directions toward th center of the cavity, as indicated diagrammatically in Fig. 11.

The more abundant the supply of calcium received from th food, the greater the development of these bone trabeculae, an *vice versa*. When this fact was first known it was cited as an instanc of *functional hypertrophy* but (at first) received only the mechanica interpretation that the ends of the bones were strengthened b abundant development of these trabeculae, as extra braces strengthe

a bridge. Now, however, they are given also a more chemical interpretation which is of very important nutritional significance. As the ends of the bones are quite vascular, they admit free circulation of the blood through them and amongst their trabeculae; and the better the trabeculae are developed the larger is the surface of contact of the blood with this trabecular bone material which "automatically" takes over the storage of any extra calcium which the blood has acquired. And this storing by increased growth of trabeculae *both* strengthens the ends of the bones and keeps them in good shape (literally) *and* increases the surface of bone salt from

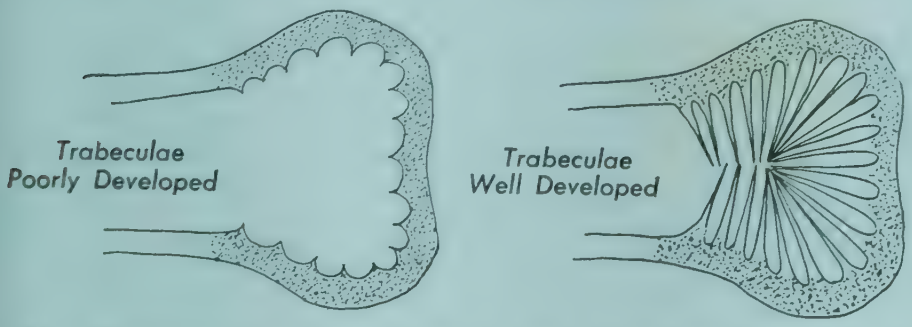

FIG. 11. Diagrammatic representations of bone trabeculae showing poor or good development according as the food-calcium intake is low or liberal.

which the blood may promptly replace any loss or expenditure of calcium which may occur incidentally to the widespread functioning of calcium in the body. Thus extra calcium received by the body is not only "passively" stored. It also adds itself promptly and effectively to the body's "working capital" of blood- and tissue-regulatory material.

Method of Determining the Amounts Required

The method which permits the most direct approach to the question of the amount of calcium or phosphorus needed in human nutrition is that of the balance experiment conducted upon the same general plan as in the case of the nitrogen balance experiments discussed in Chapter 11 in connection with the study of the quantitative aspects of the protein metabolism and protein requirement.

Inasmuch as both calcium and phosphorus after being metabolized in the body are excreted to a considerable extent by way of the intestine, and as the proportions of eliminated calcium or phosphorus leaving the body by way of the two chief paths of output (the kidneys and the intestines) may vary widely, no conclusions regarding bodily need of calcium or of phosphorus should be attempted except from experiments which include quantitative determinations in both urine and feces.

Hence, also, it would be seriously misleading to consider the amount of calcium or phosphorus found in the urine alone as a measure of the amount which the body had absorbed; or the amount in the feces as a measure of what had escaped utilization. It should be kept clearly in mind that after utilization a large part of the phosphorus and a still larger part of the calcium is likely to be eliminated through the intestine instead of through the kidneys.

Maintenance requirements of adults are studied by determining the balance of intake and output on diets of normal character but of low calcium or phosphorus content until one finds the minimum amount of calcium or phosphorus which will just permit of the maintenance of an equilibrium of intake and output of the element in question, or obtains data which permit the statistical computation of this amount.

(The small amount of calcium or phosphorus lost through the skin is not usually measured nor regarded as significant.)

In studying the calcium and phosphorus *requirements of growth,* balance experiments are also made, but here the plan which gives the most significant results is to find the intake which will support an optimal rate of storage of the element in question in the growing body. In the evolution of human anatomy and physiology there was doubtless "survival value" in the baby being born with flexible bones. But this implies being born with calcium-poor bodies which must increase their *percentage,* not merely their amount, of calcium during their growth and development. Hence there is an accentuated need for calcium (and in lesser degree for phosphorus) over the need for body-building material in general. Thus the calcium and phosphorus requirements of growth are *relatively* higher than the

protein requirement of growth. And unless the food furnishes the relatively liberal calcium intake required for optimal calcium retention, the growing body will remain calcium-poor for an unduly long time.

In pregnancy also, optimal * retention (rather than merely equilibrium) is the logical guide to the estimate of the best nutritional intake.

The Calcium Requirement

Calcium is very unevenly distributed in the body, over 99 per cent of the total amount normally being in the skeletal system. It is also very unevenly distributed among the staple articles of food, many of which are extremely poor in calcium, while milk contains it in relative abundance; and green leaves (except of the Goosefoot family) are also good sources. A food supply may appear liberal and varied, and yet, unless milk or green leaf vegetables (or both) are well represented, it may be calcium-poor. Hence the "ordinary mixed diet" of Americans and Europeans, at least among dwellers in cities and towns, is probably more often deficient in calcium than in any other chemical element.

In adults there may be a long-continued loss of calcium without the appearance of symptoms because the losses from the blood and soft tissues may be replaced by calcium withdrawn from the bones. Bauer, Aub, and Albright (1929) and also Cannon (1939) have explained how, in the organism which has been well supplied with food-calcium, the above mentioned bone trabeculae (Fig. 11) serve as a calcium reserve, readily drawn upon whenever there is, or tends to be, a loss of calcium from the blood and soft tissues.

* In this book, the adjective *optimal*, or the noun *optimum*, is intended to mean literally and strictly the *best*. In most cases we do not yet know just what level of nutritional intake is best or is needed by a person in order that he may do his best. Hence *optimal diet*, or even optimal intake of an individual factor, is in most cases an *ideal* or an *ultimate goal* which we cannot yet define in precise quantitative terms. It may frequently be desirable to reiterate McCollum's teaching that in nutrition there is or may be an important difference between the merely adequate and the optimal diet but it is never desirable to call a diet optimal merely because it is better than barely adequate. Rather we should make optimal mean the best possible

The diagrammatic representations of Fig. 11 illustrate extrem cases of the difference between the poor development of thes trabeculae in the bone of an animal which has had a diet of lov calcium content and the abundant development of the correspond ing trabeculae in the bone of an animal which has had a calcium rich diet. This increased means of quick restoration of the calciun concentration of the blood can now be seen as one of the ways i which better diets improve health and longevity.

Here the present-day chemistry of nutrition, which is being de veloped in the light of modern physical and physiological chemistry and by the use (in large numbers and with strictly quantitative laboratory control) of the whole animal organism throughout its whole lifetime as an instrument of research, is now enabling us to add to the automatic regulation of our biological inheritance. For new resources of scientific knowledge and method are being de veloped for the more precise attainment and maintenance of such an internal environment as may be found most favorable for the various activities, and the duration, of our lives.

From Table 41 it may be seen that even though the original Diet 16 was adequate for normal results as judged by each of the criteria

TABLE 41. INFLUENCE OF INCREASED INTAKE OF CALCIUM

	RATS ON DIET 16 (0.2% Ca)	RATS ON DIET 162 (0.35% Ca)
Gain 28th to 56th day		
Males, *grams*	56.3 (73)[a]	71.0 (83)[a]
Females, *grams*	47.0 (107)	60.2 (116)
Age at birth of first young, females, *days*	132 (90)	111 (98)
Duration of capacity to reproduce, females, *days*	213 (101)	279 (102)
Length of life,		
Males, *days*	658 (70)	703 (69)
Females, *days*	723 (101)	746 (101)
Young borne per female	24.1 (101)	32.6 (102)
Young reared per female	12.7 (101)	20.4 (102)
Average weight of young at 28 days, *grams*	38.9 (1286)	42.4 (2081)

[a] Figures in parenthesis indicate number of cases.

here studied, a moderate (75 per cent) increase in its calcium content resulted in more rapid growth and development, higher adult vitality, and longer life. With more liberal calcium intake, some of these criteria showed even greater increase in the betterment of the life histories.

Endocrine Abnormalities Not Discussed Here

The references at the end of the present chapter will serve to put the reader who so desires in touch with the literature of the parathyroid glands and their effects upon calcium metabolism. The present text assumes a normal endocrine constitution.

Calcium Requirements of Adult Maintenance

Up to 1920 there had been published from several laboratories a total of 97 calcium balance experiments in which the calcium intakes and other conditions were such that, according to the state of knowledge at that time, the calcium output could be tentatively regarded as throwing light upon the adult maintenance needs of normal men and women. These daily outputs averaged 0.45 gram per 70 kilograms of body weight per day. Because of individual variations and uncertainties of interpretation it was suggested that this figure be increased by 50 per cent to obtain a tentative standard allowance for use in practical dietetics.

By 1937, further data had been published and all data then available and deemed sufficiently valid were collated and interpreted in a new way by Leitch (1937, 1938), who concluded that the maintenance requirement is approximately 0.55 gram of calcium per day for normal human adults regardless of body weight. Owen (1939) found an average requirement of 0.52 gram of calcium per day in older men than those of the previous experiments.

The first Recommended Allowances of the National Research Council, formulated early in 1941, provided 0.8 gram of calcium per day for the normal maintenance of either man or woman regardless of muscular activity. This was on the two bases: (1) that

muscular work makes no significant demand upon calcium metabolism, and (2) that to meet menstrual losses and provide a bodily store for use in case of pregnancy and lactation, it was held wise to recommend the same amount of calcium for women as for men notwithstanding their lesser average body weight. It has been suggested that the elderly assimilate calcium less efficiently and should have higher intakes.

There is also clinical evidence favorable to liberal calcium intake. Dr. F. J. Stare, speaking at the 1943 Conference on Nutrition in Relation to Public Health, said of his recent hospital experience: "It was a surprise to me to see that the majority of x-ray studies on adults past the age of 45 to 50 years showed considerable demineralization of bone, and one wondered if low dietary intake of calcium, or of vitamin D, over many years, might not have been prominent factors in the etiology of this demineralization."

Almost simultaneously there appeared the work of (*a*) Chu and others (1941) who found an average requirement of 0.0073 gram of calcium per kilogram of body weight or 0.51 gram per 70 kilograms per day, in experiments with six men and six women, all healthy; (*b*) Outhouse and coworkers (1941) who found an average of 0.75 gram per 70 kilograms in seven subjects; and (*c*) Steggerda and Mitchell (1941) whose nine subjects showed an average requirement of 0.67 gram of calcium per 70 kilograms per day. Both Outhouse et al., and Steggerda and Mitchell used a method (not used by the earlier investigators) of upward adjustment of the data of output in balance experiments in which output exceeds intake, which is probably the chief reason for their higher average findings, which it will be noted are both very similar to the previously-used conventional "standard" of 0.68 gram per 70 kilograms.

In order to make due provision for individual differences in calcium requirement, and because full-life experiments with animals have shown that generous margins above absolute needs are very beneficial in the long run of the life-time, the National Research Council in 1948 raised its recommended allowance for normal adult maintenance to 1.0 gram of calcium a day. By applying the "classical" statistical interpretation to the data of all of the 73 now

apparently eligible calcium balance studies made in various parts of the world, it was found that 1.0 gram of calcium a day makes sufficient provision for individual variation to cover the needs of all but about 1 part in 100 of the normal adult population.

Of the 73 cases, which enter directly into this estimate, a considerable majority were Americans and Europeans. There are reports of people, in other parts of the world, living normally on considerably less calcium than this Recommended Allowance. But further study makes it appear more probable that the most of the people believed to be living on such low calcium levels are in reality getting significant amounts of calcium from sources which we either do not know about or habitually ignore. It is true that the milk supply which is our main source of dietary calcium is very scanty in some other parts of the world, notably in the Orient, the Arctic, and among some (by no means all) of the native peoples of Africa. But in every case which has been carefully studied it appears not that such peoples thrive on less calcium, but either that they utilize sources of calcium which we neglect or that, in the course of their lifetimes, they are handicapped by their low calcium intakes. "They may get along on it but they are not as well-off in the long run" as those who get more calcium, is a recent biochemical judgment from the Orient.

Adolph finds the calcium needs of Chinese about the same as found in Americans and Europeans, and makes clear that calcium is recognized as an important nutritional problem in China; while Hoh, Williams, and Pease (1934) have shown how a typical Chinese method of cookery brings into human nutrition a large amount of the calcium of bone, practically none of which is utilized in most American households. Kelly and Henderson (1930) found the calcium requirement of African natives essentially the same as that of Europeans and Americans. In the Arctic and in parts of the Near East, Asia, and Africa much bone-calcium is utilized by simple mastication of the smaller bones and the soft ends and interiors of the larger ones. Also, in the Orient and in parts of Africa much larger use is made of green leaf foods than in America. In certain regions not only do the people eat many coarser kinds of leaves

than we do, but of the leaves which are too coarse even for them to eat, they eat the ashes. Other previously overlooked sources of calcium are the turbid drinking-waters of some regions, and the lime that is often added to the leaves and nuts that are habitually chewed in some parts of the world.

Steggerda and Mitchell emphasize the fact that even among the healthy people selected for their experiments, the individual variations of calcium metabolism were large. This is also the impression gained by other studies of individual cases. Hence, in order that an average result shall be broadly applicable, it should be the average of as many normal cases as practicable. The importance of this was not fully reflected in the 1941 and 1945 recommendations of the National Research Council which were practically based on 16 cases all from one midwestern university. The basis was greatly strengthened when a total of 73 cases of worldwide distribution was included in the study in 1948. It was found, as also noted elsewhere, that study of this broader base showed the calcium requirement to be both higher and more variable than previously supposed. The National Research Council's *Recommended Allowances* are intended to represent not average requirements (for about half the population have requirements higher than that average): rather they aim to cover the needs of not less than about 99 per cent of the normal adults of the population. This is set, as noted above, at 1.0 gram of calcium a day.

Requirements of Pregnancy and Lactation

The calcium requirements of women are greatly increased by maternity. The need of an abundance of calcium for the rapidly developing skeleton of an infant is obvious. Before birth, and normally for several months after, this demand of the child is satisfied through the mother, whose need for calcium is thus greatly accentuated.

Lusk, citing the data of Hoffstrom, emphasized the importance of diet rich in calcium for pregnant women, especially during the last ten weeks or pregnancy when the fetus, if amply supplied, is

storing calcium at a rapid rate. Blunt and Cowan summarize the results of several studies of calcium and phosphorus balances during pregnancy in their excellent book, *Ultraviolet Light and Vitamin D in Nutrition.*

Some of the most carefully performed experiments have been planned in ways which render difficult or doubtful the exact interpretation of the observed balance of intake and output, especially when one or more factors of the intake have been allowed to vary from day to day; but it is safe to say that there is now much experimental evidence in support of emphasis upon the high calcium and phosphorus requirement of pregnancy. The National Research Council Allowances recommend increasing the calcium intake during pregnancy up to 1.5 grams a day; and in lactation up to 2.0 grams a day.

Obermer (1946) concluded from his study of about 80 cases of pregnancy that the minimal safety level for daily calcium intake is not less than 2 grams in the first to seventh months, and 2.5 grams from the eighth month to term.

Calcium Requirements of Normal Growth and Development

In children, steady growth with a normally increasing percentage of body calcium requires a relatively high calcium intake; and calcium more than any other inorganic element is likely to be deficient as the result of change of diet from milk to other forms of food.

An extended series of quantitative studies of the balance of intake and output of calcium in children was published in 1922 (Sherman and Hawley). Although this antedated most of the work upon vitamin D it is known that this vitamin was provided, because, both before and during the experiments, the children were much of the time in outdoor sunshine. Thus they received vitamin D through irradiation as well as through their food. Twelve children from 3 to 13 years old were studied as to balance of intake and output of calcium and phosphorus during a period of 9 days. All the children were normal and received a normal mixed diet including a fixed allowance of 750 grams of milk per child per day. This resulted in

a nearly uniform calcium intake of about 1 gram per child per day. The calcium retention varied from 0.15 to 0.62 gram per day, increasing with the size of the child. Calculated to the basis of size, the results show fair uniformity and indicate an average daily storage of 0.01 gram of calcium and 0.008 gram of phosphorus per kilo of body weight per day in these normally growing children. In a second series of experiments three of the children who had served

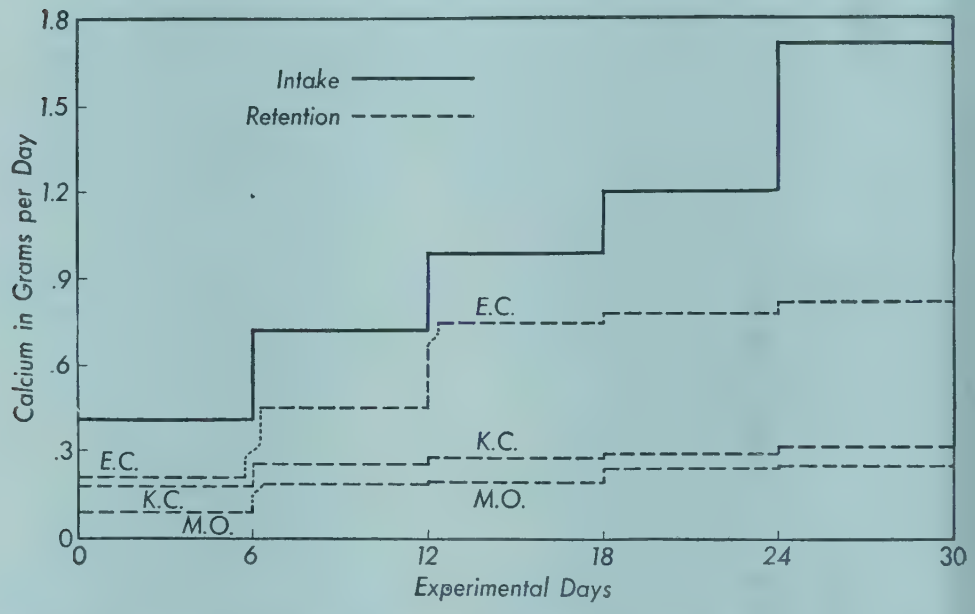

FIG. 12. Relation of calcium retention to calcium intake in three girls: E. C., aged 12 years, weight 34 kilograms; K. C., aged 4 years, weight 18 kilograms; M. O., aged 5 years, weight 21 kilograms. In each experimental period of six days, the intake was essentially the same for each child. The graphs show (*a*) the level of calcium intake of all, and (*b*) the respective calcium retentions of each.

as subjects during the first series were kept under continuous control and observation with quantitative determination of intake and output of calcium for much longer periods, the calcium intake being varied by systematic changes in the amount of milk in the diet. In part, the results are summarized in Fig. 12. At intakes of 0.4–0.45 gram of calcium per day, all showed positive calcium balances but low rates of retention. An increase of intake to 0.7–0.75 gram per day had a markedly favorable effect upon retention in all cases. A further

increase of intake to about 1.0 gram per day apparently served chiefly as insurance in the cases of K. C. and M. O.; but was evidently very directly utilized by E. C. to enable her normal process of calcification (strengthening of bones and development of teeth) to catch up with her age and size. Increases of intake above 1.0 gram of calcium per day caused only a small further increase of retention in any of these cases (Sherman and Hawley, 1922).

In the light of the fact that the previous nutritional history of the child may influence its capacity for retention of calcium and thus the level of intake required for optimal retention in the individual case, the results with these three girls may presumably be interpreted as meaning that the large capacity for retention shown by E. C. was due to a calcium-poor condition of body (as well as to her greater body weight); and that M. O., who retained lower amounts and proportions at all levels of intake, may have been so nourished as already to have a body well stocked with calcium for her age and size; while K. C. may have been in an intermediate condition with regard to her previous dietary history. Probably E. C. represents the condition of a large proportion of the children with whom one has to deal; so that the intake of about one gram of calcium per day which was found necessary to insure optimal retention in this case represents a desirable allowance to provide for individual differences and to insure against ordinary hazards due to fluctuations of efficiency in assimilation. Doubtless K. C. also represents a common type of case, able to gain even on an intake of 0.45 gram, but gaining much better on 0.75 gram and still better on an intake of 1.0 gram per day.

Outhouse, Mitchell, and coworkers at the University of Illinois have emphasized their difficulty in securing the retention by children of more than about one fifth of the calcium fed, while Jeans and Stearns of the University of Iowa find higher percentages retained by a large proportion of children, as was also the case among the New York City children studied by Sherman and Hawley.

Further and more recent evidence of individual variations in calcium metabolism comes from the balance experiments of Johnston and coworkers who found that six girls, all 13 or 14 years old and

living in like conditions with calcium intake at 1 gram a day, retained, respectively, 174, 239, 248, 279, 296, and 444 milligrams of calcium a day.

Whichever of the above viewpoints we emphasize, the main conclusion is the same: liberal calcium intake is needed by the body of the infant or child, which in order to develop to best advantage must retain a relatively larger amount of calcium than of other building materials, because normal development involves increase, not only of the amount, but also of the percentage of body calcium during growth. And intakes should be generous in order to cover the wide individual differences of need.

The Recommended Allowances of the National Research Council are: Children of all ages up to 9 years, 1.0 gram calcium per day; 10–12 years, 1.2 grams; girls of 13–15 years, 1.3 grams; 16–20 years, 1.0 gram; boys of 13–20 years, 1.4 grams per day.

The general trend of the findings of Jeans and Stearns and of Macy and coworkers, as thus far published and kindly supplemented by personal communications from the investigators to the present writer, supports high standards of calcium intake. Noteworthy also is Jeans and Stearns' repeated emphasis upon the fact that liberal allowances of vitamin D are desirable but do not justify any slackening of emphasis upon the importance of a liberal supply of food calcium.

If the objective were merely to provide for positive calcium balances in children, this could often be accomplished, as shown by Sherman and Hawley in 1922 and by Wang and coworkers in 1930, with intakes of only 0.4 to 0.46 gram of calcium per day; but to provide for such rates of retention as the newer knowledge of nutrition deems desirable, calls for an average daily intake of about 1.0 gram. The fact that a special *pre*period of extra high calcium feeding may so stock the body with calcium that for a time thereafter optimal storage will not require so high an intake, has a certain theoretical interest but little practical bearing upon our actual problem of the feeding of children as we find them. Of probably greater practical import is the evidence assembled by Leitch (1938) which seems to call for a decided advance in our standards of calcium content for the human body, and of the rates of retention needed to realize them.

From personal communications with Dr. Jeans, and independently with the late Dr. Todd, the writer has learned that without necessarily following all of the assumptions involved in Leitch's reasoning they did

concur in its main conclusions: that present knowledge calls for considerably higher estimates of the normal calcium content of the properly developed human body than have been taught generally hitherto; and that the attainment of these higher percentages requires (along with plenty of vitamin D) high standards of calcium intake for infants and for older children up to such time as they attain full "chemical maturity" and optimal adult skeletal development.

A digest and discussion of the different numerical estimates of supposedly normal calcium retentions of children at each stage of growth and development has been published (Sherman and Lanford, 3rd Ed., 1951, pages 128–133) and need not be repeated here.

Until such time as the numerical estimates of leading investigators have received their final interpretation, the Recommended Allowances of the National Research Council may well be used. In practice these are usually best realized by feeding an average of about a quart of milk per child per day; but this should not be urged too dogmatically for "every" child. An occasional child may have an idiosyncrasy which interferes; but, for general teaching and practice, an average of about a quart of milk per child per day is undoubtedly better than any materially lower average.

Calcium Intake Levels and Life Histories

We may be asked why we assume that something near maximal calcium retention is optimal, i.e., does result in greatest benefit in the long run. The answer is that we do *not assume* this: it has been found by scientific research covering entire lifetimes and successive generations of large numbers of animals with ample controls. Obviously, an experimenter who wishes to study the life-time effects of different diets must use a subject whose natural life cycle is sufficiently shorter than his own to permit him to see the experiment through. Also, the species chosen to furnish the subjects which are to serve as our "deputies" in experiments designed to throw light upon the problems of human nutrition must be sufficiently similar to us in their nutritional chemistry. The laboratory rat is well qualified in both these respects for the study of the effects of different levels of calcium intake upon permanent nutritional wellbeing.

And it may now be stated in the light of findings of hundreds of full-life, successive-generation experiments with laboratory rats, that the liberal levels of calcium intake, at least twice that of minimal adequacy, yield the best results in adult vitality, length of life, and vigor of offspring (Sherman and Campbell, 1935; Lanford and Sherman, 1938; Sherman, Campbell, and Lanford, 1939; Van Duyne, Lanford, Toepfer, and Sherman, 1941).

The question, What happens when the growing body gets only a lower level of calcium intake?, is best answered by experiments with rats whose bodies can be actually analyzed when desired, as de-

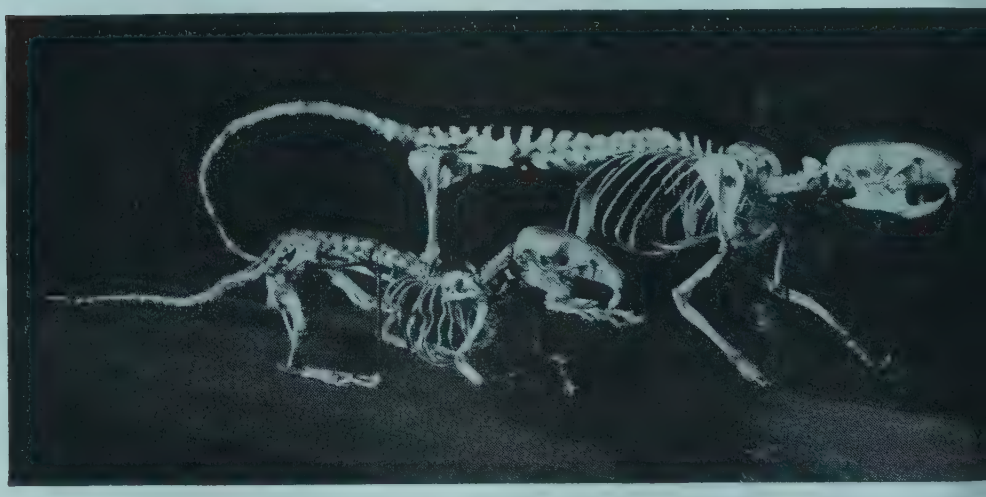

FIG. 13. Twin brothers showing effects of different diets. See text. (Courtesy of the *Journal of Biological Chemistry*.)

scribed in several of the papers listed at the end of the chapter (e.g., Sherman and MacLeod, 1925; Campbell, Bessey, and Sherman, 1935).

For simplicity of illustration and convenience of discussion, either typical individual cases or the average data of series may here be cited; but it will be understood that in all cases conclusions are based upon consistent experience with large numbers of animals under carefully controlled conditions.

In one case, twin brother rats, alike at the beginning of the experiment, were placed: one on a diet of meat, wheat, and milk; the other upon a diet of the same meat and wheat but without the

milk. The former grew and developed normally, while the latter showed some stunting of growth and markedly poor skeletal development because of his less adequate diet (Fig. 13). In this case, the relative calcium contents of the two diets were approximately as 100:14. This difference in calcium contents of the diets was doubtless the chiefly significant factor in this experiment.

In another case twin sisters were mated with twin brothers, making two pairs in all respects alike at the beginning of the experiment and having exactly the same hereditary background. Both families were fed wheat-and-milk mixtures, but in one case in the propor-

FIG. 14. Cousins from families on different diets. See text.

tion by weight of 2 parts ground whole wheat to 1 part of dried whole milk, in the other case in the proportion of 9 parts of ground whole wheat to 1 of dried whole milk. The difference in calcium content was again the most significant difference between the two diets, but was not so pronounced in this case and correspondingly the effect was slower to appear. The original experimental animals showed only inconspicuous differences, and both families reared offspring; but in the second generation (continued on the same diets as their respective parents) the difference in size and skeletal development (Fig. 14) was very similar to that which had appeared in the first generation of the experiment noted in the preceding paragraph. This is but one of many cases in which it has been found

that *observations extended through more than one generation may be required in order to ascertain fully the influence of a difference in the food supply.* In this case the calcium contents of the two diets were approximately as 100:37. Here also, as noted, calcium was doubtless the chiefly significant, though not the sole variable, factor.

On diets differing only moderately in composition (namely: Diet A, five sixths ground whole wheat to one sixth dried whole milk; Diet B, two thirds ground whole wheat to one third dried whole milk),* rat families have thrived generation after generation, both diets being adequate and supporting normal nutrition (Sherman and Campbell, 1924), yet Diet B with its higher proportion of milk and consequent higher calcium content resulted in a more favorable rate of calcification of the growing bones so that chemical analyses of the bodies of representative animals at a given age showed, for example, 0.7 per cent of calcium in those on Diet A, and 0.9 per cent of calcium in those on Diet B. Inasmuch as Diet B was richer than Diet A in fat-soluble vitamins as well as in calcium, numbers of parallel experiments were made in which Diet A was separately enriched, on the one hand with fat-soluble vitamins by addition of cod liver oil; on the other hand, with calcium by addition of calcium lactate. The results (Table 42 based on experiments of F. L. MacLeod) showed that Diet A had already contained enough fat-soluble vitamin to enable the body to make use of the available calcium, and that what was needed here was a more liberal intake of calcium itself.

TABLE 42. AVERAGE CALCIUM CONTENTS OF 90–DAY–OLD MALE RATS ON DIFFERENT DIETS (F. L. MacLeod)

FOOD OF EXPERIMENTAL ANIMAL	PERCENTAGE OF CALCIUM IN BODY
Diet A	0.70
Diet B	0.90
Diet A plus cod liver oil	0.69
Diet A plus calcium lactate	0.92

Results at four intake levels (experiments by Dr. L. E. Booher) yielded the results shown in Fig. 15.

* In both cases distilled water was furnished *ad libitum* and sodium chloride (purified table salt) in the proportion of 2 per cent of the weight of wheat consumed.

Dr. C. S. Lanford studied more intensively the influence of a difference in the calcium content of the food upon the calcium content of the body from the end of infancy to full maturity. The results of the chief series of such analyses are shown numerically for both sexes in Table 43.

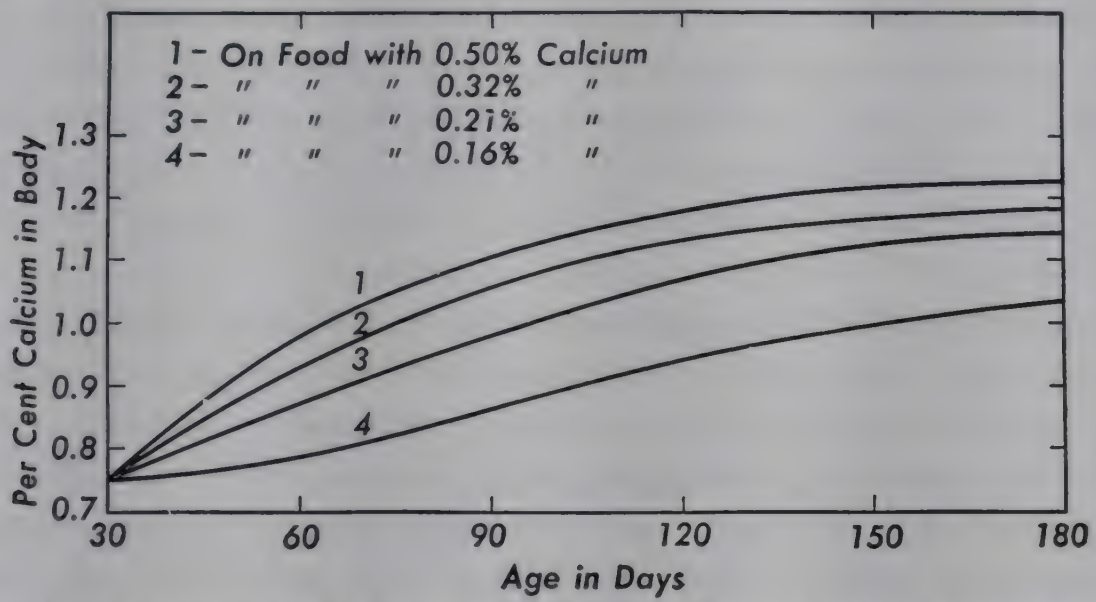

FIG. 15. Percentages of calcium in the bodies of young rats in relation to age and to the calcium content of the food. In nutritive factors other than calcium the diets were of the same composition. (Courtesy of the *Journal of Biological Chemistry*.)

TABLE 43. CALCIUM CONTENT OF THE BODY AS INFLUENCED BY AGE AND BY FOOD (C. S. Lanford)

Age	Ca in Food	Body Weight	Calcium in Body	
Days	*Per Cent*	*Grams*	*Grams*	*Per Cent*
MALE RATS				
28	0.20	39	0.279	0.715
60	0.20	102	0.692	0.677
90	0.20	173	1.262	0.726
180	0.20	260	2.651	1.023
28	0.64	40	0.385	0.956
60	0.64	117	1.128	0.971
90	0.64	172	1.765	1.026
180	0.64	282	3.137	1.113
FEMALE RATS				
28	0.20	40	0.300	0.748
60	0.20	95	0.641	0.676
90	0.20	134	1.109	0.830
180	0.20	167	1.999	1.195
28	0.64	41	0.384	0.949
60	0.64	106	1.113	1.049
90	0.64	150	1.748	1.163
180	0.64	190	2.472	1.305

Experiments with Different Calcium Intakes Continued Throughout Entire Lives

Full-life experiments have been made on two plans: (1) As the comparison of Diet A with Diet B (above) where an origin diet of natural foods was increased in calcium content by increasi the proportion of a calcium-rich food (milk) so that the extra ca cium was studied in the presence of its natural accompaniment (2) By stepwise enrichment of the basal Diet A by calcium co pounds only, or, as often expressed, by calcium as sole experiment variable.

Several hundreds of experiments extending from infancy to natur death have been made with rats on Diet A, averaging about 0. per cent, and Diet B with about 0.34 per cent of calcium (a naturally occurring enrichments of protein, vitamin A, and rib flavin). In addition to the gains on Diet B which we have alrea noted, the lengths of adult life were also improved by 10 per ce

When calcium only was added to Diet A, and only in such propo tion as to bring the calcium content of the dry food mixture to t same 0.34 per cent as in Diet B, the females again showed improv breeding records, but their average length of life was not signi cantly increased as was that of the males. Did this mean (*a*) th the females had less ability than the males to profit by extra c cium, or (*b*) that they had invested theirs in the production of mo and better offspring? The answer was sought in two ways, a with like results: *unmated* females did make as good gains in lo gevity on extra calcium as did the males; and when the calci content of the diet was again increased the females were enabl *both* to support the demands of increased reproduction *and* enjoy longer lives themselves.

With calcium intake at presumably optimal levels, and this t sole variable, both males and females increased their lengths of l by 11.5 and 13.8 per cent respectively over the records of strict parallel animals on minimal-adequate calcium-intake levels.

Here under conditions of quantitative laboratory control wi large numbers of experimental animals of the species chosen as b

tted to serve as combined instrument of precision and "deputy" or us in experimentation designed to throw light upon problems of uman nutrition. are findings which clearly show that nutrition nks with heredity as a factor in determining the length of life.

The infant mortality was decreased by the increased percentage f calcium in the family diet, which makes all the more impressive e increase in the adult life expectation (Sherman and Campbell, 935). Undoubtedly, with human beings too, a liberal surplus of lcium above actual need brings important improvement of life istory even for people of already healthy families.

It seems important to emphasize strongly the fact that on diets of irly good nutritive value, but containing less than the liberal nount of calcium which is needed for the optimal retention of lcium during growth, there may be normal gains in height and eight and every appearance of good health as indicated by care- l and thorough physical examination, yet the body (even though ining calcium) may be calcium-poor because the *rate* of calcium tention is not as high as is needed to enable the body to increase s percentage of calcium as rapidly as the *best* development of nes, and teeth, and general stamina require.

Undoubtedly a considerable proportion of people may go through e without ever attaining to as high a level of calcium content of dy as is conducive to optimal life history; and osteoporosis may found at autopsy in persons who had been believed to be nor- ally nourished. Owen, Irving, and Lyall (1940) studied calcium lances and X-ray photographs of bones in 7 men about 70 years age "and in fairly good health." Four of the seven were found X-ray to be osteoporotic; and their "normal" home diets showed w calcium intakes. The subjects were then fed, successively, three perimental diets containing respectively 0.3, 0.6 and 1.0 gram cal- um a day. At the lowest of these levels, 3 subjects were in calcium uilibrium, 3 were in slight and 1 was in marked negative bal- ce. At the 0.6 gram level of intake, 4 out of 5 showed positive, d only 1 in 5 showed negative calcium balance. Two out of three owed increased positive balance when receiving 1.0 gram calcium day. The calcium balances might be interpreted to mean that the

subjects had in a sense "adjusted" themselves to low calcium balances while the X-rays showed that this was at the expense of the development of an osteoporosis. Other investigators have found similar conditions. Autopsies are said to show a high proportion of osteoporosis in supposedly normally nourished adults examined in mid-western medical colleges.

Does High Protein Diet Call for Increased Calcium Intake?

As briefly mentioned in Chapter 11 a diet consisting of five sixths ground whole wheat and one sixth dried whole milk (with sodium chloride and distilled water) containing an approximate average of 0.2 per cent of calcium and 14 per cent of protein, has met all the nutritional needs of rat families repeatedly for over 70 generations with no sign of decline in vigor. Unquestionably it is adequate in any normal use of the term. When its protein is increased to 24 per cent by addition of lean meat,* prepared muscle protein, or casein, growth becomes more rapid and puberty develops earlier but significant numbers of the females break down in reproduction or lactation sometimes with symptoms suggestive of calcium deficiency. Some of these high protein animals analyzed for the purpose have shown lower body-calcium content than the average for their age. These animals may be regarded in one or more of three ways: as victims of over-stimulated growth, or of calcium deficiency, or as showing protein:calcium imbalance. The incidence of this disorder (or the death-rate of females in the susceptible age range) is materially reduced when extra calcium is added to the basal diet at the same time with the extra protein. Experiments are now (1951–1952) in progress to determine as accurately as practicable the extent to which the hazard is obviated by extra dietary calcium, and the amount required for best results.

* Lean meat has been most often used, the other two forms of protein giving like results so far as tested.

The Phosphorus Requirement

Quantitative Study of the Maintenance Requirement

Study of the data of 95 presumably suitable phosphorus balance experiments upon 21 subjects, 14 men and 7 women, has shown a range of 0.52 to 1.20 grams with an average of 0.88 gram of phosphorus per 70 kilograms of body weight per day as the minimum intake which permits equilibrium. This is an average of findings as to actual requirement for normal maintenance.

The National Research Council has not set Recommendations for phosphorus.

Phosphorus Requirements of Normal Growth

In the experiments of Sherman and Hawley described above in connection with the discussion of calcium requirements for growth, it was found that optimal retention of phosphorus in the growing children of 3 to 13 years of age was not obtained until the intake reached from 1.16 to 1.46 grams of phosphorus per child per day, thus indicating that the child needs for optimal growth and development about one and one-half times as much phosphorus as is needed by a full-grown man for maintenance.

Additional studies have been conveniently summarized by Blunt and Cowan (1930).

Ca/P Ratios

Especially since the common use of diets of distorted Ca/P ratios to produce experimental rickets, there has been something of a tendency to state Ca/P ratios in connection with normal nutrition also.

The ratio of calcium to phosphorus in the body as a whole is normally increasing throughout the period of growth and calcification. In the normal adult mammal the Ca/P ratio of the whole body is a little under 2; and that of the bones is a little over 2.

Meigs and coworkers (1935), as the result of their very thorough experimental investigation of milch cows and study of previous

work, have concluded: that the percentages of calcium and phosphorus contained in the fat-free bodies of mature normal cattle are relatively constant, and the Ca/P ratio, both in the whole body and in bone, still more so; that keeping cows on low-calcium rations for long periods alters these relationships less than has often been thought. Their long periods on calcium-poor foods changed the Ca/P ratio of the whole body only from 1.9 to about 1.8, for when calcium was lost phosphorus was lost also. They held that previous reports of positive balances on low-calcium intakes and of retention of calcium and phosphorus in widely variable ratios are probably due to experimental errors. For obviously when intakes of both elements are right, the ratio cannot be wrong.

Calcium and Phosphorus Contents of Typical Foods

There are given in Table 44 the data for a limited number of typical foods to illustrate the differences in quantitative distribution of calcium and phosphorus. Data for many other foods will be found in the Appendix.

Discussion of variation of nutritive value among normal foods of a given kind is postponed to Chapter 31.

As McCollum emphasized strongly from about 1918 onward, plants tend to concentrate calcium in their leaves; phosphorus in their seeds; the roots and tubers occupying an intermediate position with respect to their calcium and phosphorus contents. Similarly among foods of animal origin, milk is rich in calcium (with an advantageous phosphorus content also), meats are relatively rich in phosphorus and very poor in calcium, and eggs are intermediate between meat and milk.

Relative nutritional availability of the calcium of different foods. As this section must be very short, it may conveniently take the form of a brief summary of a few series of experiments.

Rose studied the utilization of the calcium of carrots in calcium-balance experiments with healthy young women to whom she fed diets carefully planned to furnish just about the maintenance requirement of calcium, one half of which was in the form of carrots.

Under these conditions the calcium of the carrots was nearly as well utilized as that of milk.

TABLE 44. CALCIUM AND PHOSPHORUS CONTENTS OF TYPICAL FOODS

FOOD	CALCIUM	PHOSPHORUS	CA/P RATIO
	Mg./100 G.	Mg./100 G.	
Almonds	254	475	0.53
Apples	6	11	0.55
Asparagus	21	57	0.37
Beans, dry	148	437	0.34
Beans, snap or string	65	44	1.48
Beef, lean muscle	11	204	0.05
Carrots	39	37	1.05
Collards	250	74	3.38
Eggs	54	210	0.26
Kale	225	67	3.36
Milk	118	93	1.27
Oatmeal (oats)	53	405	0.13
Oranges	38	23	1.65
Oysters	70	150	0.47
Peaches	8	22	0.36
Peas, dry	72	388	0.19
Peas, fresh green	26	128	0.20
Potatoes	11	56	0.20
Sweetpotatoes	30	49	0.61
Tomatoes, seeds removed	6	20	0.30
Turnip greens	260	58	4.48
Watermelon	7	12	0.58
Wheat, entire	54	374	0.14

Rose and MacLeod made a similar study of the utilization of the alcium of almonds. When almonds furnished about 73 per cent of he total food calcium, the calcium of the almonds was almost as ell utilized as the calcium of milk or of carrots; but when the monds were made to furnish about 85 per cent of the total food alcium the efficiency of utilization of the calcium of the almonds as somewhat less.

Fincke's experiments were made with rats, so that the retention f calcium could be studied over a much longer segment of the life-ycle, and could be determined by the direct analysis of the body the end of the experimental period. The experimental animals ere fed, through a period during which normal growth and de-

velopment involves a relatively large increase in the calcium content of the body, on carefully planned diets in which calcium was furnished alternatively by the different foods to be compared. It was found that the calcium of milk was well utilized, that of kale almost as well, while the calcium of spinach was utilized to only a very slight extent, if at all.

Costa (1947), using the technique and diet of Fincke, found in experiments with rats calcium utilization factors of 14 for spinach, 84 for lettuce, 81 for cress, and 76 for cabbage.

From this and other similar investigations (Fincke and Garrison; Kao, Conner, and Sherman; Kung, Yeh, and Adolph; Mallon, Johnson, and Darby; Speirs; Tisdall and Drake), it now appears clearly that the calcium of celery-cabbage, Chinese-cabbage, collards, kale, leeks, lettuce, rutabaga leaves, tendergreen, and turnip greens is well utilized in nutrition; while that of spinach and New Zealand spinach is almost if not entirely useless, probably because of the oxalic acid contained in these (and doubtless other) leaves of plants of the Goosefoot family (Chenopodiaceae).

Shields, Fairbanks, Berryman, and Mitchell (1940) reported that under the conditions of their experiments (with growing rats) the calcium of carrots, lettuce, and string beans was, respectively, 85, 80, and 74 per cent as available as the calcium of milk.

Fincke (1941) has found the calcium of broccoli and of cauliflower to be about as well assimilated as that of kale, these three foods ranking almost with milk in the availability of their calcium.

Outhouse and coworkers (Breiter *et al.*, 1942) found highly variable results in studying the utilization of the calcium of carrots by seven human adults.

It may be emphasized that availability or utilization experiments, whether with man or other animals, should be made in rather large numbers and in a strictly side-by-side manner before one is justified in attempting quantitative comparison of the findings.

Several investigations have shown good utilization of the calcium of finely-ground bone, and of bone ash.

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Chapter 15

IRON AND COPPER IN FOOD AND NUTRITION

Importance in Normal Nutrition

The amount of *iron* contained in the body is small—only about one part in 25,000 of the body weight or about a tenth of an ounce in a full-grown healthy person—yet its functions are prominent and fundamentally important:

(1) It is an essential element of the chromatin substances which in some sense preside over the most vital activities of the cells.

(2) It is also an essential element of the hemoglobin which is the outstanding substance of the red cells (erythrocytes) of the blood, and thus of the blood's property of taking up oxygen in the lungs and carrying it to all the tissues in which oxidation occurs.

(3) Iron likewise is an essential element of the myoglobulin (muscle hemoglobin) which is closely related, chemically and functionally, to hemoglobin, acting with it to regulate oxidation-reduction (redox) systems.

(4) Both iron and copper participate further in tissue oxidation-reduction reactions through the catalytic actions of cytochromes and cytochrome oxidases which have been estimated by Schultze (1947) to "mediate about 90 per cent of the energy transfer associated with the aerobic phase of tissue respiration."

(5) And finally, iron is an essential part of other highly important body enzymes.

In addition to these five active forms of iron, the body may also carry a reserve in the form of ferritin, a non-diffusible substance from which iron can be liberated as needed for hemoglobin formation.

Widespread and continuous as are the functions of iron com-

pounds in the tissues, yet the greater part of the iron which the body contains is normally concentrated in the blood. If we accept the estimates that, in round numbers, the blood constitutes about 7 per cent of the body weight and contains 55 to 60 per cent of the body iron, then obviously the concentration of iron is about eight to nine times as high in the blood as in the body as a whole; and about twenty times as high in the blood as in the average of the other parts of the body. And very much the largest part of the iron in the blood is present as an essential constituent of the hemoglobin of the red blood cells. Hence an iron-poor body tends to be anemic; and, while anemias may be due to many other causes as well as lack of iron, the studies of the adequacy of iron supply in nutrition have been closely interwoven with the studies of anemia, of hemoglobin formation and regeneration, of the history and functioning of the red cells or corpuscles of the blood.

Copper is in some way involved in the process of hemoglobin formation. While its action is catalytic in the sense that it does not appear as a constituent of the hemoglobin formed, yet this action of copper in hemoglobin formation and regeneration * is now regarded as part of the normal nutritional process. Hence copper is now counted among the nutritionally essential elements; and the study of iron and copper in nutrition, of the nutritional anemias and hemoglobin formation and regeneration, are so closely interdependent as to be scientifically inseparable.

Blood Formation and Anemias

Formation of red corpuscles. It is generally taught that in the normal adult, red cells are *formed* only in the bone marrow. After an average life of about four months, they are destroyed, either by fragmentation in the blood stream or by phagocytosis by endothelial cells, especially in the spleen. As the corpuscles are broken down, the hemoglobin is split into an iron compound and bilirubin.

* To a considerable extent, some of the parts of a catabolized hemoglobin molecule are normally used over again in making new hemoglobin; there is no sharp distinction between hemoglobin formation and regeneration.

The bilirubin is carried to the liver by the blood stream and there secreted in the bile.

A large part of the products of breakdown of hemoglobin, both of the iron-containing and of the bilirubin fractions, is retained in the body and re-used in hemoglobin formation.

The number of red cells and percentage of hemoglobin is slightly higher in men than in women, and these also vary somewhat with age. The newborn child has about 40 per cent more red cells and hemoglobin per unit volume of blood than has the adult. During the first three months of life there is usually a decrease to about 65 per cent of the adult values. Between the ages of three and six months this usually rises to about 75 per cent of the adult values, after which there is continued gradual increase until the adult condition is established.

As contrasted with a normal condition of the blood, the term anemia is generally applied either to a deficiency of hemoglobin, or of red corpuscles, or both.

The relationship between the hemoglobin and the number of red cells (corpuscles) is important in classifying various types of anemia. It is expressed either directly as *mean corpuscular hemoglobin* (usually in millionths of a milligram) or as the *color index:*

$$\text{Color index} = \frac{\text{Hemoglobin (\% of normal)}}{\text{Number of red cells (\% of normal)}}.$$

Recent research on anemia, considered from the nutritional point of view. As an outgrowth of studies upon the significance of substances secreted in the bile, Whipple and his coworkers were led to an extended investigation of the regeneration of hemoglobin after experimental hemorrhage, and the influence of different foods upon the rate of such regeneration in dogs maintained upon a standard basal diet. Liver was found to be effective in this connection. Minot and Murphy then tried liver clinically in a very different kind of anemic condition, namely, in pernicious anemia. Here also it proved effective, and this clinical finding has prompted a very interesting nutritional research, one of the results of which has been the finding in liver extract of a specific curative substance. This is

the vitamin B_{12} which in the present book is best studied at its chemically logical place in Chapter 21.

Simultaneously, there has been active nutritional investigation of still another type of anemia, an anemia produced experimentally in laboratory animals by the long-continued feeding of iron- and copper-poor food, especially during the period of growth. To make clear the differences between the different types of anemia and to guard against confusion in their correlations with the normal metabolism of iron (and copper) there follows a present-day classification of the anemias with discussion of the nutritional considerations involved.

Classification and Nutritional Discussion of the Anemias

(N.B.—In the minds of some readers, the question may arise whether this section is too medical for this book, and whether we might omit mention of anemias not due to dietary deficiency. The answer is that while this book leaves medical responsibilities strictly to the medical profession, yet every student of nutrition and food chemistry should be educated to the fact that anemias may arise from other causes as well as from dietary deficiencies, and that to discriminate is not always simple.)

Modern classifications of the anemias differ somewhat in form. Sometimes the basis of classification is the change in the blood picture, and sometimes the grouping is in accordance with the cause of the departure from the normal. Of the latter type is that of Haden (1935), on which the following is based:

Haden's Classification of the Anemias According to Cause

I. Increased blood loss.
   (*a*) Mechanical loss (hemorrhage): see below.
   (*b*) Accelerated destruction in the body: as in certain types of infections and poisoning, and certain familial abnormalities.

II. Depressed blood formation.
   (*a*) Depressed bone marrow function: due to poisons, infections, nephritis, tumors of the bone marrow.

(*b*) Deficiency of specific substances: see below.
  (1) Deficiencies of substances involved in hemoglobin formation (hypochromic anemias).
  (2) Pernicious anemia type.

From the point of view of strictly nutritional aspects the two outstanding types are therefore:

I. Those due to loss of blood (hemorrhage).
II. Those due to deficiency of some substance or substances essential to hemoglobin or red cell formation.

Among deficiencies we distinguish
(*a*) Those of substances which are concerned with the formation of hemoglobin—iron, copper, and certain organic factors.
(*b*) Those having rather to do with the formation and development of the red corpuscles as such—"the specific substance which is effective in curing pernicious anemia" (vitamin B_{12}).

I. Anemias due to hemorrhage. The first effect of a large loss of blood is to reduce the volume in circulation. The bulk is rapidly restored by the absorption of fluid from the tissues, which dilutes the hemoglobin and red corpuscles. In the regeneration of the blood, new red cells are formed more rapidly than hemoglobin. The reserves of pigment-forming substances in the body are easily exhausted, so that while the stroma or framework of red corpuscles is restored, their hemoglobin content is deficient. In this state, the blood is said to have a low color index. The result of chronic loss of small amounts of blood is quite similar.

Regeneration of the normal blood in these cases appears to be chiefly a problem of restoring the hemoglobin. Whipple has shown that this is markedly influenced by the diet. Both organic and mineral factors are important. The former are sufficiently supplied by liver and kidney.

Although these experiments led to the use of liver in pernicious anemia, it is now believed that the action in the two conditions is different and involves different factors, one favoring the formation of hemoglobin (after loss of blood) and the other, of the red corpuscle stroma (recovery from pernicious anemia). Extracts rich

in the factor which cures pernicious anemia are much less potent in the anemia of hemorrhage.

In human beings, on ordinary diets, iron is practically always effective in promoting recovery from the anemia of hemorrhage.

II. Anemias due to deficiency of some substance or substances. Here the deficiency may be the fault of the food or of digestion or absorption.

Deficiency of hemoglobin-forming substances. The number of red cells may or may not be reduced, but characteristically there is a low color index.

The relatively long continued feeding of exclusive milk diet to rabbits or rats results in an anemia which seems to be due to deficiency of both iron and copper. If the milk accidentally contains enough copper, this experimental milk anemia can be cured by giving iron; but otherwise both iron and copper are needed, the iron as an essential constituent of the hemoglobin, and the copper to promote the process of hemoglobin formation.

This method of experimentation has been much used as a means of testing the availability of the iron and copper contents of foods, or the *hematopoietic properties* of food regardless of whether iron, copper, or some unknown factor is the limiting factor in the particular case.

It is still an open question to what extent such experiments bear upon the human problem. From the standpoint of the relative life cycles, it takes a month in the life of a child to be equivalent to a day in the life of a rat; and so it would be necessary to confine a child absolutely to an exclusive milk diet for years in succession in order to parallel the experimental nutritional anemia induced as just mentioned in rats.

Moreover, it has been found that the above-mentioned decrease of hemoglobin which takes place in the first three to six months of human life is physiological rather than pathological and is not prevented by giving iron. The consensus of medical opinion is that only a small proportion of the cases of anemia which occur among infants below six months of age can be attributed to shortage of iron, or copper, or both.

During the last months of intrauterine life relatively large amounts of iron and copper are normally stored in the liver of the fetus; so that the body at birth is relatively rich in both of these elements. Correspondingly, milk shows relatively low iron and copper content; but as the iron (and presumably the copper) of milk is readily assimilated and economically used in the body, the fact that the infant (born iron-rich) is chiefly nourished with milk for several months, does not involve any serious danger of a nutritional shortage of iron. As insurance against any slight danger of such shortage, the foods introduced early into the diet of the child are chosen with reference partly to their iron content, as well as largely with reference to their vitamin values.

Recent research also indicates that a considerable part of the iron which disappears from the *circulation* of the infant during the physiological decrease of hemoglobin content is retained in the body stores available for resynthesis into hemoglobin with the growth of the child.

Around the age of six months, the child needs an adequate supply of iron to maintain the increased hemoglobin formation, and if, as often occurs in premature infants, twins, or the offspring of anemic mothers, the body stores are low, the iron content of the food then becomes a matter of importance.

Idiopathic hypochromic anemia is the term now applied to an anemia characterized by a low color index and not associated with hemorrhage or any other known cause. It occurs most frequently in women about thirty to fifty years of age and is usually associated with a subnormal acidity of the gastric juice. In many cases the diet has been shown to be of low iron content, and it is thought that failure to absorb the iron (because of the defect in the gastric juice) may be equally important in bringing about a relative shortage of iron in the body. The losses of iron in menstruation may also be a factor. Liberal intakes of iron produce rapid improvement.

Pernicious anemia group. The nature of the deficiency here is quite different from those described in the previous paragraphs. The substance specifically effective in pernicious anemia has to do with the production and development of the body or stroma of the red corpuscle rather than with its hemoglobin.

This type of anemia is characterized chiefly by deficient formation of red cells, which are greatly reduced in numbers and vary in size and shape, with large cells so prominent that the term *macrocytic* anemia is often used to describe pernicious anemia and other anemias presenting the same blood picture. There is a relative abundance of hemoglobin, with a high color index, large stores in the muscles and excess of iron deposited in the liver, spleen, and kidneys.

A characteristic feature of pernicious anemia is deficient gastric secretion.

Castle has shown that the necessary factor is produced in the digestion of beef by normal gastric juice. Neither beef nor normal gastric juice alone had any effect on pernicious anemia, but an incubated mixture of the two had the same action as liver.

Since this type of anemia occurs in patients with deficient gastric secretion, he concluded that the normal person is able to produce the protective substance by the action of the *intrinsic factor*, present in the gastric juice, upon the *extrinsic factor* furnished by the food. Individuals with pernicious anemia lacked the intrinsic factor and were therefore unable to form the essential protective substance. This hematopoietic material * was, however, supplied preformed by the feeding of liver or kidney. The feeding of stomach is also effective, whether because of containing the same hematopoietic material as liver, or because it supplies the intrinsic factor. After absorption the active substance is stored in the liver, which explains the relative richness of liver as a source of this material.

The extrinsic factor of Castle has now been shown to be present in milk, egg, beef muscle, wheat germ, and autolyzed yeast. In coagulated milk this factor remains with the curd.

Fully purified preparations of the liver substance effective in pernicious anemia have been chemically identified and named vitamin B_{12} (see Chapter 21).

Among other types belonging to the pernicious anemia group are:

* The term *hematopoietic* is or may be somewhat loosely applied to whatever results in blood regeneration in a given case. Hence according to circumstances it may be applied to the combination of the intrinsic and extrinsic factors, or to either of them, or even to some other substance needed for hemoglobin formation.

Cases observed in India in which the deficiency is that of the extrinsic factor; cases of lack of intrinsic factor through destructive disease or surgical removal of the stomach; sprue (see Chapter 21), and certain other diseases of the intestines, in which the active material apparently is not absorbed in adequate amounts.

It may happen that the same patient has both the hemoglobin deficiency and the pernicious type of anemia.

Anemia of pregnancy may be of either of the main types described above, or a combination of them. During pregnancy there is likely to be deficiency of gastric secretion at the same time with withdrawal of body iron for the fetus, thus increasing the danger of both the hypochromic and the pernicious type of anemia. The storage of iron in the fetus appears to vary rather widely with the amount in the mother's diet, and the completeness of its absorption.

Interpretation

From the foregoing, which attempts to summarize a modern classification of the anemias with only brief running comment upon nutritional relationships, it will be seen that the three lines of research which have recently attracted much attention from students of nutrition, namely, the work upon the anemia resulting from hemorrhage, that upon anemia induced by exclusive milk feeding, and that upon pernicious anemia, really deal with different conditions.

The condition studied by Whipple and his coworkers is that which follows the withdrawal of blood from an otherwise normal and well-nourished body. It is to be kept in mind that what the body has to replace in such experiments is not any one substance, merely, but whole blood. Under the conditions of these experiments it is found that regeneration of hemoglobin tends to be the dominant feature in the situation. This is probably due at least in large part to the use in these experiments of a basal ration which provides for other nutritional needs while throwing upon the particular food (or drug) under investigation the special function of providing the material needed for hemoglobin formation.

In striking contrast with the condition existing in the experimental anemia of hemorrhage, is the condition found in "true" or typical pernicious anemia, where there is no lack of hemoglobin as such (and presumably no lack of the substances which enter into its synthesis in the body), but rather a lack of something involved in the production of the stroma—the framework of the red corpuscle which holds the hemoglobin and carries it throughout the body. The substance wanting in this type of anemia probably does not enter into the hemoglobin molecule at all; but is a highly specific substance essential to the (process of) formation of red blood cells * without which the hemoglobin itself cannot function effectively in the service of the body as a whole. Plainly the situation here is so far different from that of hemorrhage that we must be careful to avoid the confusion which is apt to arise from the fact that these two conditions are both called anemia.

A third type of anemia recently much studied is that induced experimentally by feeding young animals *exclusively* upon milk for an abnormally long time. The resulting condition is different from either of the two which have just been discussed.

It is important to keep in mind that the three kinds of anemia which have recently been so actively and successfully investigated are really three quite different states.

Recommended Allowances of Iron

There is growing evidence that, especially in the case of men, and, to a lesser extent, in other age and sex groups, the amount of iron actually *needed* on a diet which is otherwise good, may be distinctly lower than was formerly thought. Moreover, problems of experimental technique and interpretation (discussed in Sherman and Lanford's *Essentials of Nutrition*) make the exact determination of

* A frequent feature in the blood of pernicious anemia is the decreased proportion of *young* red blood cells which are called reticulocytes in contradistinction to the fully developed erythrocytes. The rapid appearance of reticulocytes in the blood following the feeding of liver, or a potent liver extract, is an even earlier evidence of the success of the treatment than is the increase in the total red cell count, and, therefore, reticulocytes figure largely in the literature of this subject.

minimum need especially difficult in the case of iron. For the purposes of the present text, therefore, it is considered sufficient to reproduce the recommendations of the National Research Council, with the comment that these allowances appear generous when compared with present evidence on actual requirement.

RECOMMENDED DAILY ALLOWANCES OF IRON

(National Research Council)

For men,	12 mg.
For women,	12 mg., increasing to 15 mg. in pregnancy and lactation
For children,	
under 1 year,	6 mg.
1–3 years,	7 "
4–6 years,	8 "
7–9 years,	10 "
10–12 years,	12 "
13–20 years,	15 "

No specific recommendations are made for copper intake, though it is commented that, "the requirement for copper for adults is about 1 to 2 mg. daily. Infants and children require approximately 0.05 mg for each kilogram of body weight. The requirement for copper is approximately one-tenth that for iron. A good diet normally will supply sufficient copper."

Iron (and Copper) in Foods

Figures for the iron contents of foods are given in Table 66 of the Appendix; and values for copper in certain foods will be found on pages 630–632 of the Seventh Edition of this book. It may be pointed out further that, according to current (1951–52) United States government regulations, bread and rolls sold as "enriched" must contain at least 1.8 mg. of iron per 100 grams; while for "enriched" flour, cornmeal, macaroni products, etc., the minimum is 2.9 mg. of iron per 100 grams.

Summary

Whipple has said that a monument erected to commemorate developments in iron metabolism would be, indeed, a Tower of Babe

and that the confusion is largely due "to an attempt to apply findings n one type of anemia to a totally different type of anemia."

In the practical problem of the adequacy of the food supply to neet the nutritional needs of the body, the iron content of the food s doubtless a more important consideration than its copper content because the environment as a whole, with its accidental additions of copper to our food and drink, will almost always furnish us at least as much copper as we need.

It was also the opinion of the conference on treatment of diseases of the blood, held at the Cornell University Medical College and reported in the *Journal of the American Medical Association* in July 1940, that in only a small proportion of clinical anemias is there advantage in the addition of copper to the iron prescription.

There is no doubt that the "enrichment" of breadstuffs, the "restoration" of cereals, and the growing use of green vegetables have together materially increased the iron content of the American dietary as a whole. And at the same time we have found our normal need to be less than we had supposed. So, as regards the iron problem in nutrition, recent years have brought us reassurance.

The present-day student of nutrition is entitled to the enjoyment of such moments of reassurance when they are encountered, for our study of several other factors will necessarily leave us with the impression that new knowledge has increased the burden of nutritional responsibility.

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Chapter 16

IODINE IN NUTRITION: SIMPLE GOITER AS A NUTRITIONAL PROBLEM

Natural Distribution of Iodine and Its Relation to Goiter

Iodine is one of the essential chemical elements of the human body, although it constitutes only about one part in three million parts of the body weight. Sea salt, and therefore the spray which evaporates in the air at the seashore, is relatively rich in iodine; contained as iodide. And as this sea-salt dust is carried inland by the wind it gives iodide to the rain-water, the soil water, the soils, and the crops of nearby regions.

Also, some rocks contain enough iodine to yield significant amounts of soluble iodide in their natural weathering. But there are other regions whose water supplies do not come from such rocks, and which, because of too great distance or because of intervening mountains, receive practically none of the air-borne salt-dust of evaporated sea-spray. These may contain in their waters and in the crops grown on their soils too little iodine to meet the needs of normal nutrition. The people who grow up in such regions show a high proportion of simple goiter, which is primarily an enlargement of the thyroid gland resulting from its being obliged to function without an adequate iodine supply. Of course, these glands may also become infected. When the ordinary foods and drinking waters of a region are too poor in this element, the small amount of iodine needed to prevent goiter can be supplied by adding iodide to the drinking water or the table salt; by incorporating some iodine compound into tablets with food or confectionery; by the use of sea foods; or by consuming in or with one's food a small amount of any of various sea plants not otherwise used as food, which have been found to contain iodine.

The recent tendency in discussions of the variations in iodine contents of waters and foods has perhaps been to lay more emphasis upon the chemical nature of the rocks in the mountains, and less upon the spray of the sea, than was customary in previous years. There may even be a significant difference in this respect between the rocks on the two sides of the same mountain range.

The regional variations of incidence of goiter were recognized both in Europe and in Asia before its relation to iodine deficiency was understood.

As a striking example of an iodine-poor region, McCollum and Simmonds cite Keith's study of the Pemberton Valley in the Pacific Northwest. There, many of the men, most of the women, and almost all the children had enlarged thyroids. The domestic animals also showed great prevalence of goiter. As a result of providing iodine, either as iodized salt or as sea food, most of the trouble has now been rectified.

It should be borne in mind that, while lack of iodine is often, and probably usually, the dominant factor in producing simple goiter, infections arising from contaminated water supplies or generally unhygienic conditions may also play an important part, as has been particularly emphasized by McCarrison. In a more recent discussion of goiter, however, McCarrison has emphasized the fact that the more nearly perfect the nutritional intake, the more nearly perfect will be the thyroid gland, both as to its size and structure and as to its functioning. The possibility that under unusual circumstances other factors in the diet may play a part in causing goiter is discussed, along with the relation of iodine, by Greer (1950).

McClendon and Williams correlated the iodine content of drinking water with the prevalence of goiter in four zones in the United States as follows: With 0.01–0.1 part of iodine per billion of water, the goiter rate was 15–30 cases per 1000 of population; with 0.015–1.2 parts per billion of iodine, 5–15 cases; with 0.06–9.0 parts per billion, 1–5 cases; with 1.4–10 parts per billion the rate was less than 1 case per 1000. They also produced enlarged thyroids by deprivation of iodine in controlled laboratory experiments upon rats.

McClendon, Barrett, and Canniff (1934) have furnished another

illustration. They find the average iodine contents of Minnesota potatoes to be: 85 parts per billion for the eastern, and 226 for the western section of the State. Correspondingly the records show an incidence of 19 per thousand of simple goiter among men drafted in 1917–1918 in the eastern and only 7.5 per thousand in the western section.

The same lack of iodine which causes goiter may also cause myxedema which is attributed to an insufficiency of thyroxine, a nutritionally active iodine compound formed in the thyroid gland.

Iodine and the Thyroid Gland: Thyroxine

In 1895, Baumann discovered iodine in the thyroid gland. Subsequent work showed that this iodine is intimately connected with the activity of the thyroid. The characteristic physiological effects of administration of thyroid gland appear to be directly proportional to the iodine content of the gland administered.

In 1914, Kendall isolated from thyroid a pure crystalline substance containing about sixty-five per cent of iodine, which substance he named *thyroxine* and showed to be capable of exerting the characteristic effects of thyroid upon metabolism.

In 1926, Harington determined the detailed structure of thyroxine. Very interesting in this connection is the isolation by Foster (1929) of 3,5 di-iodotyrosine from thyroid.

Whether thyroxine circulates and functions in the body in its free state or in combination with protein, as in thyroglobulin, or in both ways, is still a subject of discussion. Heidelberger and Pedersen (1935), considering thyroglobulin to be the principal protein elaborated in the thyroid gland and, "as appears probable, . . . the actual thyroid hormone," have studied it by modern physicochemical methods and find evidence that its molecular weight is of the order of 675,000 or around twenty times as large as that of insulin. As it is difficult to picture the direct diffusion of such large protein molecules through cell membranes these investigators suggest that the thyroglobulin may be deposited on the surface of a cell, and then either exert its action by means of its loosely bound thyroxine groups, or be

hydrolyzed by the proteolytic enzymes of the cell with a gradual liberation of free thyroxine at the surface of the cell whose activity it catalyzes. Such a concept helps us to understand the relatively slow and long action of the thyroid hormone as compared with most of the other hormones which have so far been investigated.

Kendall (1929) and Harington (1933) give full accounts of the laboratory work of isolating and of synthesizing thyroxine and many interesting illustrations of the use of the products thus obtained in the clinical treatment of diseases which had resulted from the inadequacy of the thyroid secretion (goiter, myxedema, cretinism). The thyroid utilizes simple iodide in the preparation of thyroxine perhaps directly and perhaps in part through intermediate substances such as that isolated by Foster (1929). Normally the small amounts of iodides contained in food and drink furnish sufficient iodine both for the proper functioning of the thyroid gland and the production by it of the hormone needed to regulate metabolism throughout the body. As briefly indicated above, when the amounts of iodine furnished by the food and drink are insufficient, the thyroid gland becomes enlarged. It is now generally agreed that this is the cause of most simple goiter, and that simple goiter is, therefore, in its origin, an iodine-deficiency disease. For photographs both of an adult and of a child recovering from thyroxine deficiency, see Sherman and Lanford's *Essentials of Nutrition.*

Seaweeds and sea water have long been the familiar sources of iodine. In regions where the people live largely upon sea food, and the atmosphere and drinking water are constantly receiving the iodine-containing salt spray blown in from the sea, the intake of iodine is presumably adequate and in such regions goiter is very rare; but in regions too remote or too mountainous to receive significant amounts either directly or indirectly from the sea, goiter is much more common, as in the Great Lakes region and much of the Northwest of the United States, in parts of Switzerland, and in several other parts of the world.

Marine and Kimball, reasoning that if the prevalence of goiter in such regions is due to a lack of iodine it should be preventable by giving iodide to children at the age at which goiter ordinarily begins

to develop, obtained permission to try their now classical experiment in the public schools of Akron, Ohio. Here as many as volunteered of the pupils of the ages known to be most susceptible to goiter were given small doses of sodium iodide dissolved in drinking water twice weekly over a period of a month and repeated twice yearly. As is well known, this experiment was strikingly successful. In only 5 cases among over 2000 treated was there any enlargement of the thyroid gland when iodide was taken, while in a similar number of children of the same age in the same region not taking iodide about 500 showed enlargement during the same time. Hence it would appear that 99 per cent of the simple goiter of this region could be prevented by iodide, if means for its universal administration could be found. This work of Marine and Kimball, first published in 1917 and subsequently (1921) confirmed and extended, has focussed attention upon the relation of iodine supply to goiter both in this country and abroad. In three Swiss cantons where an attempt was made to give iodide to all school children during the three years 1918–1921, the incidence of goiter was diminished during the three years from 87 per cent to 13 per cent.

The general subject of the functioning of the thyroid gland has been well reviewed by Marine (1935) who emphasizes the importance of the interrelations of the thyroid with other organs both of internal and of external secretion, but adds that as to our knowledge in this field only a good beginning has been made. According to Marine, the fact that the principal function of the thyroid is to increase oxidative processes in the body indicates that all bodily activities are influenced by the state of functioning of the thyroid gland and *vice versa*. He estimates the normal iodine content of the (20 to 25 gram) human thyroid at an average of 10 to 15 mgms., with a maximum of 20 to 25 mgms. The thyroid gland retains iodine even during fetal life, the amount acquired depending largely upon the iodine intake of the mother.

Marine considers (1935) that the substance actually secreted by the thyroid is probably iodothyroglobulin (commonly called thyroglobulin) but that thyroxine appears to be the active part of the thyroglobulin molecule, natural and synthetic thyroxine both hav-

ing essentially the same effect as natural thyroid secretion, and showing the maximum degree of the effect somewhat more quickly.

Marine suggests that the exact way in which thyroxine increases oxidative processes in the cells may perhaps be not yet fully known; but that there is considerable evidence that epinephrine (adrenine, adrenaline) and thyroxine may work together in this process. There are also reasons for believing that thyroxine is always present and acting, though in differing degrees according to the amount secreted by the gland, whereas the throwing of epinephrine into the circulation by the adrenal glands may be essentially an emergency response. In this case, the two would act together in times of markedly accelerated oxidation rate, while thyroxine alone may suffice to maintain oxidation at such rates as obtain in times of relative quiescence.

Marine also cites observations which suggest that diets rich in proteins and fats increase the rate of discharge of thyroxine and that "thyroid activity is more necessary in the oxidation of fats and proteins than of carbohydrates." In this same review, Marine (1935) gives considerable attention to the interrelations between the activity of the thyroid and the activities of other glands.

Iodine Content and Requirement of the Body

Evidently the body has a more or less definite nutritive requirement for iodine. Qualitatively this requirement might be stated as a sufficient amount of iodine to meet the daily losses from the body and maintain such store as is needed to provide for the manufacture and the distribution throughout the body of sufficient amounts of thyroxine (thyroid hormone) to support a normal rate of physiological activity.

According to Marine, if the iodine content of the thyroid is maintained above 0.1 per cent *of its solid matter*, goiter does not develop. In the thyroids of healthy soldiers killed by war wounds Zunz found an average of about 0.05 per cent iodine *in the fresh substance*, which, as the glands weighed about 26 to 30 grams, amounts to about 15 milligrams of iodine in the thyroid gland of a full grown healthy man. According to the estimates of Kendall and Plummer

the rest of the body may be expected to contain about 10 milligrams more of iodine, probably chiefly in the form of thyroxine, which (whether free or in combination with protein) has been distributed by the thyroid and is serving to control metabolism in all the active tissues of the body.

Thus the full grown healthy man is estimated to contain in his 70 kilograms of body a total of about 25 milligrams of iodine, equivalent to 1 part in about 2,800,000 parts of body substance or less than 0.00004 of one per cent of the body weight.

The difficulty of quantitative determination of iodine in the extremely minute amounts in which it occurs in most plant and animal tissues must be expected to render somewhat slow and uncertain the working out of satisfactory data on the iodine values of foods and the quantitative requirements of the body for iodine at different ages and under different conditions. Fellenberg estimated that the normal human adult requires about 0.000014 gram of iodine daily and that, when larger amounts are furnished by the food and drink, an easily mobilized reserve store of iodine is built up in the body. It is doubtless through the building up of such a body store of iodine that the administration of iodide for two weeks to one month twice yearly by Marine and Kimball proved so effective in preventing the development of goiter throughout the year as above noted. Adolph and Whang (1932) estimated the per capita iodine intake in the non-goitrous coastal region of East China at 0.000032 to 0.000066 gram per day (32 to 66 mcg.). As yet there cannot be said to be a consensus of expert opinion as to the amount of iodine required in normal nutrition. Two of the reasons that this has not yet been established probably are: (1) that the reserve which the body tends to carry can be so conserved that negative balances do not soon become perceptible; and (2) that the actual amounts of iodine involved in human nutrition and contained in ordinary foods are so small that the "personal equation" in the use of even the best available analytical methods becomes a relatively large factor and, therefore, different investigators working upon the same absolute amounts may nevertheless reach different quantitative findings.

The Food and Nutrition Board of the National Research Council

estimates that a desirable daily intake of iodine is 0.002 to 0.004 mg. per kilogram of body weight, or a total of 0.15 to 0.30 mg. for the adult. They state that "this need is met by the regular use of iodized salt; its use is especially important in *adolescence* and *pregnancy*."

The prevention of goiter in regions whose soils and waters are very poor in iodine is a nutritional problem whose immediate solution may depend not so much upon detailed knowledge of iodine contents of foods as upon the use of methods which lie largely in the province of the health officer.

The above-noted success of Marine and Kimball in preventing the development of goiter through the giving to the school child of a little iodide in drinking water, naturally suggested the addition of iodide to public water supplies, and this has been done in several communities.

Two other methods have also received favorable attention: the administration of iodine compounds in chocolate tablets or some equivalent palatable form, and the addition of a small proportion of sodium iodide to table salt intended for household use.

Olin (1924) described the state-wide survey of thyroid enlargement and the preventive measures undertaken in Michigan in part as follows:

"The survey showed to us the extent of our problem and it suggested the state-wide remedy. The unvarying relationship between the percentage of thyroid enlargement and the iodine content of the water supplies gave added proof that simple goiter was a nutritional rather than a medical problem. Making good the iodine deficiency in the state's food supply would solve the problem. The question was how to do this:

"Treatment of water supplies was promptly ruled out. This method has been tried and found not entirely effective in several cities. Added to that, it would affect only the cities at best, and our problem was as much rural as urban.

"Chocolate iodine tablets are already in use in the school systems of several cities of Michigan. Their effectiveness is beyond question, but again, this way of solving the problem applies best to cities with well organized schools and continued educational propaganda among parents. It has the added disadvantages of failing to reach two important groups —the expectant mother and the preschool child.

"It was clear to us that a condition that was the result of a state-wide

food deficiency could best be remedied by supplying that deficiency through an inexpensive and universally used food stuff. Salt was, of course, the logical medium to be chosen, since crude salt commonly contains iodine. Representatives of all Michigan salt manufacturers were therefore invited to Lansing and at an enthusiastic conference the agreement was reached whereby all companies would put on the Michigan market, on May 1, 1924, a new 'iodized' table salt."

Turrentine of the United States Department of Agriculture has emphasized the possibility that it may prove advantageous to give iodine in a form less readily soluble and less rapidly absorbed into the circulation than is a simple iodide solution, and has advocated the use of kelp and particularly preparations of the species *Macrocytis pyrifera* which he finds to be particularly rich in iodine; and Adolph and Whang (1932) find the *Laminaria religiosum* of the Chinese seacoast to be also a rich source of iodine.

We have here discussed the relation of iodine supply to the occurrence and prevention of simple goiter. It is not the function of this book to discuss the treatment of already developed goiters. Wherever the disease exists it should be under medical care. In adenomatous goiter, for instance, there are medical reasons for not giving added iodide; and partly for this reason there has recently been a tendency to reduce the proportion of iodide recommended for addition to the table salt for general sale in goitrous regions.

The use of salt containing one part of sodium (or potassium) iodide to from 5000 to 200,000 parts of sodium chloride, as a *preventive,* is simply a matter of normal nutrition. The added iodide is not to be regarded as a drug but rather as restoring the table salt to something like its natural composition—the ordinary table salt of today being the product either of the selection of exceptionally pure sodium chloride mineral (halite) or of an artificial refining process, and being thus in a sense denatured. Thus in the case of Charleston, West Virginia, cited by McCollum and Simmonds, the community up to 1898–1900 had used relatively natural salt from local salt wells and had been practically free from goiter; but between 1898 and 1900 this was almost entirely displaced by the now familiar refined table salt and thereafter a larger proportion of the school girls began to show enlarged thyroids.

Kimball (1928), after years of practical experience as a physician giving special attention to goiter, stated explicitly that there is no real danger in the use of iodized salt for the prevention of goiter and emphasized the importance of such prevention both in childhood and in pregnancy.

More recently, the American Medical Association's Committee on Foods has repeatedly approved iodized salt for general use, and in at least one announcement it is noted that the brand of salt under consideration in that particular case contained also a little sodium carbonate to stabilize the added iodide. There seems now to be no reasonable doubt that iodized salt can be made a satisfactory means of supplying the nutritional need for iodine. Obviously, then, to the extent that iodized salt is utilized, the natural iodine content of foods becomes a matter of abstract scientific interest rather than of practical dietetic anxiety. Yet so long as there remain regions in which the iodine content of the drinking water is low and the use of iodized salt is not practically universal, cases may sometimes occur in which the adequacy of the iodine intake depends upon the choice and the source of the food.

Iodine Contents of Foods

In the case of iron or copper the great difficulty of quantitative studies of nutritive requirement lies in the extreme smallness of the amounts concerned in the body's daily intake and output. In the case of iodine this difficulty is still greater. As a matter of fact, the quantities of iodine in most foods are so small that the analytical methods used in examining foods for iodine have not usually been very conclusive even as to whether iodine is present or absent. Doubtless in many cases iodine has been reported absent in foods which really contained it, but in quantity too small to be found by the analytical method employed. It is sometimes stated or implied that ordinary foods do not furnish iodine and that unless sea foods are used the iodine intake will depend entirely upon the drinking water; but this is too pessimistic a view. McClendon has analyzed several kinds of foods from a number of regions and has seldom if ever reported the absence of this element from any natural plant or animal product.

Nevertheless the data in Table 45, compiled from the work of several investigators, will serve to illustrate (1) the wide variation of iodine content in foods, (2) the divergence of findings of different workers upon the same food, and (3) the tendency of foods from goitrous regions to show less iodine than food of the same sort from non-goitrous regions. Note that the figures are only *milligrams* of iodine *per metric ton* of dry matter in the food, or *parts per billion.*

TABLE 45. IODINE IN FOODS FROM GOITROUS AND NON-GOITROUS REGIONS: PARTS PER BILLION OF DRY MATTER

KIND OF FOOD	GOITROUS REGIONS	NON-GOITROUS REGIONS	AUTHORITY
Wheat	1–6	4–9	McClendon
Oats	10	23–175	McClendon
Carrots	2	170	McClendon
Carrots	—	507	Okla. Agr. Expt. Sta.
Lettuce	—	618	Okla. Agr. Expt. Sta.
Potatoes	85	226	McClendon
Cabbage	—	776	Adolph and Whang
Cranberries	—	26–35	Morse
Asparagus	—	946	Okla. Agr. Expt. Sta.
Radishes	—	994	Okla. Agr. Expt. Sta.
Tomatoes	—	379	Okla. Agr. Expt. Sta.
Milk	265–322	572	Remington and Supplee
Butter	140	—	McClendon
Sea foods:			
Codfish	—	5,350	U. S. Bureau of Fisheries [a]
Conch	—	1,140	U. S. Bureau of Fisheries [a]
Crabmeat	—	1,460	U. S. Bureau of Fisheries [a]
Flounder	—	1,480	U. S. Bureau of Fisheries [a]
Oysters	—	1,800–3,500	U. S. Bureau of Fisheries [a]
Red Snapper	—	1,440	U. S. Bureau of Fisheries [a]
Salmon	—	570–2,200	U. S. Bureau of Fisheries [a]
Shrimp	—	1,100	U. S. Bureau of Fisheries [a]
Cod liver oil	—	7,670	U. S. Bureau of Fisheries [a]
Cod liver oil	—	3,000–13,000	Holmes and Remington

[a] Coulson, 1935.

Fellenberg also found more iodine in the food of the non-goitrous regions of Switzerland than in the food of the Swiss regions where goiter is prevalent. In general Fellenberg finds the iodine content of grains and legume seeds to range from 8 to 64 parts per billion; of fruits from 6 to 120; of nuts up to 200; of vegetable oils 30 to 95; of cod liver oil about 5000 parts per billion. Chile saltpeter contained

in a fresh sample 192,000 and in an old sample 49,000 parts—such large quantities as to have some effect upon the iodine content of the soils and ground waters where this nitrate is liberally used as fertilizer. Mineral waters examined by Fellenberg showed results ranging from 11 to 6000 parts of iodine per billion. Fellenberg and his associates have also discussed, much more fully than space permits here, the distribution and circulation of iodine in organic and inorganic nature; and more recently other investigators have published quantitative work of similar purport.

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Chapter 17

ASCORBIC ACID (VITAMIN C)

Discovery and Chemical Identification

Five centuries or so ago scurvy was one of the most prevalent diseases in Europe. In fact one writer then suggested that all diseases might be considered as different modifications or outgrowths of scurvy. European medical literature does not show how long the people had suffered from this disease. It is known to have been one of the afflictions of the Crusaders; and when Vasco da Gama, near the end of the fifteenth century, made his historic voyage around the Cape of Good Hope, he reported the death by scurvy of nearly two thirds of his crew. In 1535 Cartier, obliged to winter in Canada, lost a quarter of his men by scurvy, and nearly all the others were ill with it. From the natives they learned that decoctions of the twigs and leaves of certain trees served to cure and prevent the disease. Captain Cook and his men escaped scurvy by making a regular practice of stocking their ship with the fresh fruits of all the shores that they visited, giving the same careful attention to this as to the replenishment of their drinking water.

What the Europeans suffered from scurvy during their exploration and colonization of the New World was compensated for in two ways: they learned the antiscorbutic values of fresh vegetables and citrus fruits, so that as early as 1720 Kramer wrote of these as the sure preventive; and they brought the potato from America to Europe where, as potato culture became common, scurvy became relatively rare.

It continued, however, to afflict sailors on long voyages, and Lind as surgeon of the *Salisbury,* finding himself obliged to treat an epidemic of scurvy with only scanty supplies, decided to give the

available oranges and lemons to two of his twelve patients while others received respectively, cider, cream of tartar, elixir of vitriol, or other drugs which had been recommended by different medical writers. The account of this experience which Lind published in 1757 had, therefore, much of the character of a report upon a controlled experiment. The men receiving the oranges and lemons were completely cured, those who had cider showed improvement, while the other patients gradually became worse.

In 1841, Budd explicitly ascribed the *antiscorbutic property* possessed by certain foods to a *definite substance* which, he wrote, "it is hardly too sanguine to state, will be discovered by organic chemistry or the experiments of physiologists in a not far distant future." Ninety years later, Budd's prediction was fulfilled by C. G. King, employing the methods both of organic chemistry and of experimental physiology in his researches at the University of Pittsburgh.

King's chemical identification of vitamin C, quickly confirmed by Szent-Gyorgyi in 1932 and soon followed by the synthesis of the substance, made this the first of the vitamins to be conclusively established as a substance of known molecular structure; as it may also be considered to have been the first to be clearly postulated as a definite chemical entity (by Budd, in 1841).

In the meantime, however, the vitamin concept had been independently developed through studies of normal nutrition and of beriberi, so that when the antiscorbutic substance was formally incorporated in the vitamin scheme of 1920 by Drummond, it was given only third place in the vitamin alphabet, McCollum having already designated a fat-soluble A and a water-soluble B.

To avoid monotony, we shall here make about equal use of the two names, vitamin C and ascorbic acid.

Molecular Structure of Ascorbic Acid and Its Chemical Behavior *in vitro*

As no name indicative of its structure and of convenient length was forthcoming, the designation ascorbic acid has been generally accepted as being distinctive and historically appropriate.

In molecular structure, ascorbic acid is closely related to the monosaccharide sugars, and to several of them. This fact makes possible the synthesis of ascorbic acid from different carbohydrate starting-points. Thus the substance, after King's identification, soon became relatively inexpensive, and so is widely available as research material, as well as for therapeutic use.

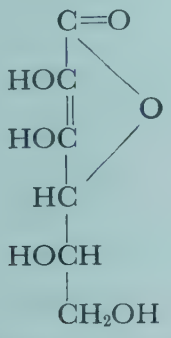


FIG. 16. Vitamin C (Ascorbic Acid): 2, 3 dienol-*l*-gulonic acid lactone.

Even before the substance had been isolated it was known to be readily oxidized. Its oxidization is much more rapid in alkaline than in acid solutions, and is markedly catalyzed by even traces of copper. Although ascorbic acid is, in general, the most labile of the known vitamins, and its destruction under ordinary conditions is chiefly due to oxidation, it is not autoxidizable within the normal pH range of plant and animal tissues. Rather, the rate of its oxidative destruction depends both upon its oxidation-reduction potential and a delicate interdependence of pH and the concentrations of other factors. (See King, pages 328–330 of *The Vitamins, 1939.*)

As more fully summarized by King (l.c.), the oxidation-reduction potential of ascorbic acid is such that it is reversibly oxidized to dehydroascorbic acid by a wide variety of organic compounds and of inorganic reagents.

Among the former are the indophenol dyes, and among the latter is iodine. Both these have been employed quantitatively for the determination of ascorbic acid, in many plant and animal tissues including foods.

The method most generally adopted is that depending upon the

carefully controlled oxidation of ascorbic acid to dehydroascorbic acid by the phenol-indophenol dye with the corresponding reduction of the latter to its leucobase, as developed by Bessey and King and modified for still greater accuracy. (Cf. Bessey, pages 354–364, *The Vitamins, 1939.*)

Because of the possible presence in urine of small amounts of some substance or substances of oxidation-reduction potential so similar to that of ascorbic acid as to involve the danger of being counted with it, Roe has developed a method for blood and urine analysis in which ascorbic acid is determined not by oxidation but by means of a carbonyl condensation reaction. This new method is of much value in clinical research.

Destruction of Vitamin C by Catalyzed Oxidation

The conservation of the vitamin C values of foods depends upon protecting the vitamin from oxidation not only by the exclusion of air in the ordinary sense, but also by freeing the material from dissolved air, by the avoidance of copper and other such catalysts as might gain entrance from even brief contact with utensils as well as the longer contacts with containers, and by the maintenance of a low temperature, and of an acid reaction. It is also to be remembered that the oxidation hazards are not all from without; for if cabbage juice (to mention a single instance) is carefully acidified to the same pH as tomato juice, and the two are treated with exactly the same precautions in all the respects above mentioned, the vitamin C value will still fall more rapidly in the juice of the cabbage than in that of the tomato, because of the natural presence in the cabbage of substances of higher oxidation potential than in the tomato.

Numerical Expression of Vitamin C Values

Vitamin C values and requirements are most satisfactorily expressed in terms of milligrams of the actual substance, and this plan is followed in the present book. The now rarely used Interna-

tional Unit of vitamin C is defined as 0.05 mg. of the actual substance.

Nutritional Chemistry of Ascorbic Acid (Vitamin C)

Prominent as is the oxidation-reduction behavior of ascorbic acid *in vitro*, the exact way in which this property functions nutritionally in the animal body has not yet been so fully and clearly worked out as to permit of simple concise statement, though King and coworkers are closely approaching that goal. Meanwhile, the nutritional functions of vitamin C which have thus far been most clearly established are (*a*) its relationship to the formation and maintenance of intercellular cement substance, (*b*) its significance in the safeguarding of the body through the blood, and (*c*) its role in regulating the rate of cellular respiration.

Wolbach (1937) has especially emphasized the relation of this vitamin to the body's intercellular material. He points out that shortage of vitamin C may thus become the underlying cause: (1) of hemorrhages which may occur anywhere in the body; (2) of profound changes in the structure of the teeth and gums; (3) of changes in the growing ends of the bone with beading and other deformities which formerly were mistaken for rickets; (4) of the falling apart of bones due to loss of supporting cartilage; (5) of enlargement of the heart and damage of heart muscles; (6) of degeneration of muscle fibers generally, causing extreme weakness and even death; (7) of the anemia due to destruction of blood-forming cells in the bone marrow and loss of blood by hemorrhage; (8) of loss of calcium through degeneration of the bone matrix, the bones sometimes becoming so soft that they break spontaneously; and (9) of a degeneration of the sex organs and other glandular tissues.* Evidently then, vitamin C, the antiscorbutic substance, is much broader in its functioning than simply to prevent scurvy. When scurvy shall have been banished into ancient history, people will doubtless continue to study the functioning of vitamin C.

* Recent work suggests a close relationship of vitamin C to the functioning of the adrenal glands. (*See* Review, 1950.)

Deficiency of ascorbic acid leads to an increased metabolic rate, s measured by oxygen consumption, both in the intact animal Fidlar, E., Sheppard, M., and McHenry, E. W., *Biochem. J.* **33,** 344 1939)) and in isolated tissue slices (Stotz, Schultze, Harrer, and Zing, 1938).

Sealock has shown that ascorbic acid is essential for normal netabolism of the amino acid tyrosine in guineapigs. Incompletely xidized tyrosine was excreted by scorbutic animals, and slices of ver from scorbutic animals showed the same loss in capacity to xidize tyrosine beyond the quinone stage. When ascorbic acid was dded to the tissue slices from scorbutic guineapigs, normal oxidation f tyrosine was resumed. This finding promises to be of far-reaching mportance as an indication of a major specific function of the itamin in cellular metabolism (Lan and Sealock, 1944) and Levine f Cornell Medical College observed a similar relationship in pre-nature infants.

Inasmuch as shortage of vitamin C may have any of such widely listributed results in the body, as indicated in the four preceding paragraphs, one's individual bodily constitution may determine how nd where the penalty will be paid for neglect of vitamin C in one's hoice of food.

The absence of scurvy does not prove that the food is supplying s much vitamin C as the body needs for its best health, vigor, and bility to resist disease. Thus in the experiments of King and Menten 1935) in which guineapigs on graded levels of vitamin C intake eceived injections of graded amounts of diphtheria toxin, it was demonstrated that the animals less liberally supplied with the vita-min, although they showed no external symptoms of scurvy, were much more sensitive to injury from the bacterial toxin at all dosages of the latter than were parallel animals on higher intakes of vita-min C.

Vitamin C probably functions in the nutritional processes of all species; those which do not need to have it in the food presumably make their own within their bodies.

Studies on the vitamin C content of animal tissues indicate that t is present in practically all tissues of the higher animals, including

those of species (such as the rat and chicken) which thrive on diets containing no demonstrable amount of vitamin C, as well as in species (man, guineapig, and monkey) which are susceptible to scurvy. *The relative distribution in the body* is similar in both groups of animals, being highest in glandular tissue (in particular the adrenals, pituitary, corpus luteum, and young thymus) and lowest in blood and muscle; but the concentrations are higher in those species which can synthesize vitamin C (and are therefore presumed to maintain tissue stores at or near optimal level) than in human subjects who have received an ordinary or conventional diet. In a series of analyses of human tissues obtained at autopsy, Yavorsky, Almaden, and King (1934) found wide individual variations and concluded that, in approximately one fifth of the people represented, the tissue concentrations of vitamin C were distinctly below what is conducive to optimal health.

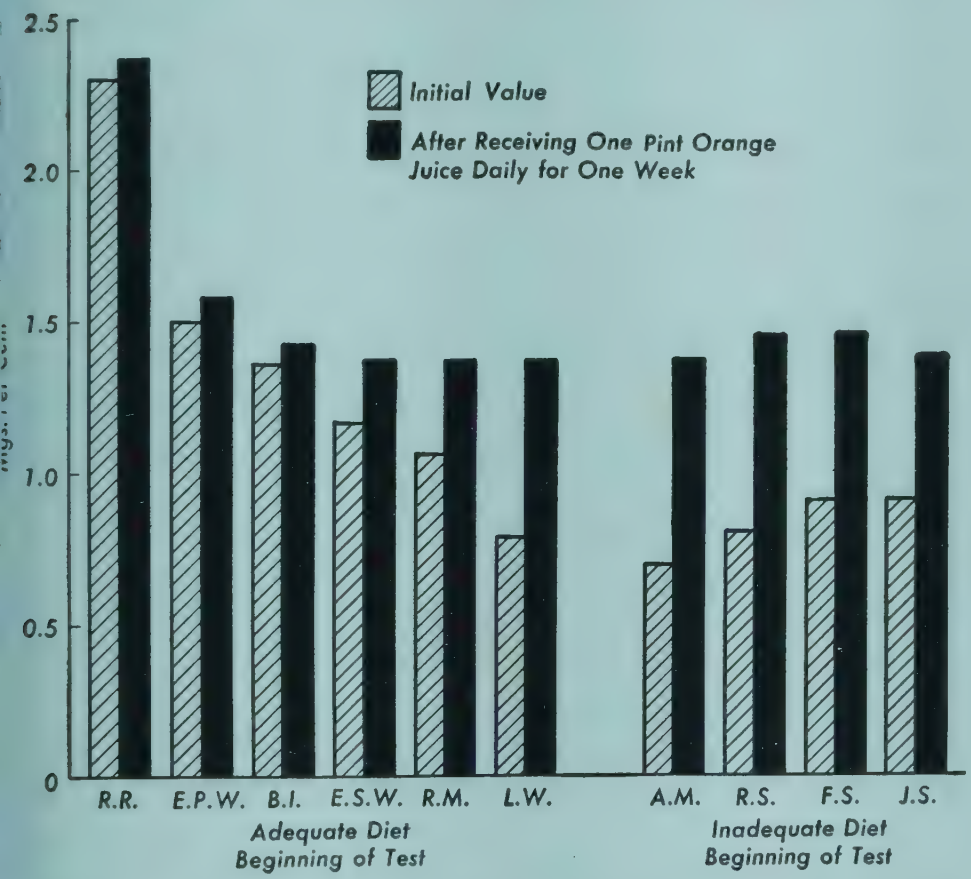
The relation of vitamin C to the teeth deserves discriminating consideration. Enthusiastic reports of several years ago, that attributed to this vitamin a specific value in the combat of dental caries, met considerable skepticism. The evidence on this point is conflicting and the question is still debated. The inconclusive state of the caries problem, however, should be distinguished from some other aspects of "the tooth problem" on which evidence is much clearer.

It had earlier been found that vitamin C is highly essential to good internal structure of the teeth; and Boyle, Bessey, and Wolbach (1937) definitely showed the value of this vitamin in the prevention of the systemic type of pyorrhea. Thus the soundness both of the teeth themselves and of their supporting bones and gums is importantly dependent upon the amount of vitamin C supplied by the food.

King and coworkers (1940) found that guineapig teeth, in addition to being very sensitive to structural abnormalities when subjected to mild vitamin C deficiencies, are injured in much greater degree when the animals are injected with diphtheria toxin. To withstand the effects of toxin injections, as shown by retention of normal tooth structure, vitamin C intake levels must be from two

o three times higher than are required when the animals are not ubjected to the toxin. Thus there was a distinct advantage to the issues gained from having more than a bare protective allowance of itamin C.

Concentration levels in the body are influenced both by the rate t which the body receives and the rates at which it destroys and



IG. 17. Vitamin C content of the blood as influenced by the diet. The six people whose records are shown on the left had all received apparently adequate diets prior to the beginning of the test. Note, however, the considerable individual differences among them, doubtless due mainly to their differing choices of food. The four people to the right, who were known to have had low dietary intakes of vitamin C, all show low levels of vitamin C in the blood at the time of the initial determinations, results of which are indicated by the crosshatched columns. After generous intakes of this vitamin for one week each person showed a gain, and those whose blood levels had been low are all now well up in the normal range. (From Farmer and Abt in *The Vitamins, 1939* by permission of the American Medical Association, publishers, and by courtesy of Dr. C. J. Farmer and the Milbank Memorial Fund.)

excretes the vitamin. Notwithstanding the solubility of vitamin C, its concentration may vary widely among the tissues and body fluids of the same individual. It has been most frequently determined in the blood plasma, with the concentrations stated in "milligrams per cent" which in this case may, without appreciable error, mean either per 100 grams, or per 100 cubic centimeters or milliliters.

An interesting illustration from the work of Farmer and Abt is shown in Fig. 17. From this it will be seen: (1) that one week of feeding a pint of orange juice daily in addition to the previously customary diet brought up into the normal range all of their subjects whose initial blood values were subnormal; and (2) that this more liberal daily allowance of vitamin C in the diet also appreciably increased the level of concentration of vitamin C in the blood plasma (and probably in the body generally) of those whose initial levels were already within the normal range.

That even in the case of so soluble, diffusible, and labile a substance as vitamin C the body habitually carries a higher concentration when it receives a higher intake is a fact of much importance to our growing appreciation of the influence of one's daily food habits upon one's *internal environment.*

This flexibility of the body's internal environment (as contrasted with its previously postulated *fixité*) is very important both theoretically and practically; but of course there are both practicable limits and individual differences.

It will be noted that nine of the ten persons represented in Fig. 17 attained a fairly constant plasma-concentration of between 1 and 1.5 "mg. per cent" when their intakes of vitamin C were liberal. Similar results have been found in a large majority of the individuals studied by these and other investigators. (In the few exceptional cases the plasma level may be either lower or higher than the general average.)

Saturation

Saturation is often used as a technical term to express the condition in which the body shows little disposition to add further to its concentration of vitamin C. The level of intake required to maintain

saturation, in this sense, is naturally higher than that needed to support a plasma-concentration of, say, 0.7 mg. This latter—or in some cases (Crandon, Lund, and Dill, 1940; Lund and Crandon, 1941) even a much lower–level may be consistent with health over relatively long periods; but the present consensus of opinion of those who have especially studied the subject favors the maintenance of at least an approximation to the "saturation" value.

This is partly to provide for frequent emergencies. Thus the Harris group usually obtained notably smooth and clear-cut results in their studies of the relation of output to intake; but also found that common infections (even a light cold) lowered the urinary output of ascorbic acid, presumably because of an increased rate of destruction of the vitamin in the body.

Similar evidence of increased destruction of vitamin C in the body during infections has been reported by several independent investigators and for several different diseases. More of the vitamin is then needed if serious shortage in the body is to be avoided.

King and his coworkers have further shown (as already briefly noted) that shortage of vitamin C lowers the body's resistance to bacterial toxins, and that this lowered resistance may become serious before there is any external evidence of scurvy lesions. They also found that injection of bacterial toxins caused rapid and marked depletion of vitamin C in the body.

There is some evidence that bacterial toxins accelerate vitamin C losses from human tissues also. For example, Macy and her associates observed that children who showed no evidence of scurvy after long periods on relatively low intakes rapidly developed scorbutic symptoms when infections were superimposed upon the "latent" deficiencies. After recovery from the infections, the symptoms of scurvy gradually disappeared. (Hamil, B. M., Reynolds, L., Poole, M. W., and Macy, I. G., *Am. J. Diseases Children* **56**, 561 [1938].)

Todhunter and Robbins (1940), studying the amount of vitamin C required to maintain tissue saturation in normal adults, found variation not only from person to person but in the same person at different times. Thus one of their subjects was apparently saturated on a total daily intake of 90 mg. at one time but at another

time needed 110 mg. However, when the data of repeated tests wer averaged, their three subjects (normal adults) all showed require ments of 1.6 to 1.7 mg. of ascorbic acid per kg. of body weight pe day for maintenance of body saturation. In these subjects "satu rated" blood plasma values, determined 24 hours after a test dos of 400 mg., ranged from 1.41 to 1.66 mg. per 100 ml. of the bloo plasma. A uniform total daily intake of 60 mg. resulted in (befor breakfast) plasma levels of 1.0 to 1.17 mg. per 100 ml., whereas level of intake twice as high (120 mg.) did not quite suffice to kee the plasma level up to 1.4 mg. throughout 24 hours. The data o these and several other experiments indicate a sort of "threshold, usually at about 1.4 to 1.5 mg. per 100 ml. of blood plasma thoug an occasional individual may show a higher threshold as indicate by "R.R." in Fig. 17.

Todhunter, Robbins, and McIntosh (1942) studied the rate of in crease of the ascorbic acid content of the blood and the duration o the higher plasma level after ingestion of a 50 mg. dose. The vitami C content of the plasma showed measurable increase in about hal an hour, reached its maximum in one and one half hours, and ha returned to the previous level in 3 to 4 hours. With larger dose the rise was greater and the effect lasted longer; but as 50 mg. i more than a third of an ordinary day's allowance, it is of interes to see that its effect on the plasma level lasted less than 4 hours. Ev dently a liberal intake more than once a day would be needed t keep the plasma level constantly high.

Comprehensive surveys of vitamin C status in college women hav been made by Brown, Fincke, Richardson, Todhunter, and Wood (1943) and by Dodds and MacLeod (1944 *b*).

Dodds and MacLeod (1944) found average vitamin C concentra tions in blood plasma of 0.67 mg. per cent in freshmen and 0.84 mg per cent in upper-classmen of the same college community.

The amount of vitamin C contained in the body, the rate at whic it is destroyed, and the rate at which it leaves through the kidney are all subject to relatively wide variations under the conditions o ordinary life; and do not depend solely upon the level of intak though they may be largely influenced by it.

The differences are at least partly individual; long ago it was observed that in a large group of children of the same age and environment, some need significantly more vitamin C than others. From their writings it would appear that Peary needed more antiscorbutic in the Arctic than did Stefansson. When the latter argues from his observation of the Eskimos that vitamin C is less important than nutritionists think, he may give too little weight to the probability that the Eskimos (and perhaps some of the Norwegians also) may be the result of a "natural selection" of people whose vitamin C requirement is lower than that of most of us.

Moreover, Hoygaard and Rasmussen offer evidence that the typical food supply of the Eskimos actually contains very considerably more vitamin C than has been supposed, largely because of the unnoticed consumption of marine algae, as well as the fact of their eating liver and other parts *immediately* upon the killing of animal, bird, or fish.

That the amount of vitamin C required to maintain a given concentration of this substance in the blood is larger for some people than for others is also being found in current studies of the status of nutrition of American college students.

Other conditions being uniform, the amount of vitamin C excreted in the urine depends upon the level of intake and the concentration level in the body. The more vitamin C the body already has, the smaller the proportion of a given test dose it will retain and the greater the proportion that will reappear in the urine.

The response to a test dose of extra vitamin C as measured by analytical determination of the increased concentration in the blood, or the increased excretion in the urine, or both—has been much studied for the sake of the indication it affords as to the bodily status or "level of vitamin C nutrition."

Here again, individual variability in vitamin C metabolism is illustrated in the very carefully controlled laboratory investigation of healthy human subjects by Todhunter and Fatzer (1940). Both before and after the addition of oranges to the dietary, their Subject A excreted more vitamin C in the urine, while showing a slightly lower concentration of this vitamin in the blood, than their Subject B. The quantitative response in urinary

output to a given increment of intake was about alike for the two subjects, indicating that they were about equally well saturated, as would have been judged from the analyses of their blood but not from the analyses of the urine.

Another excellent illustration of individual variation is that reported by Storvick and Hauck (1942), two of whose subjects, both healthy women, were prepared by saturation in the same way and then were studied as to response to successively lowered levels of intake. At each level the two subjects showed essentially the same urinary excretion but one always a markedly higher ascorbic acid content of blood plasma than the other.

Levcowich and Batchelder (1942) studied different quantitative aspects of ascorbic acid metabolism in 45 young college women, 32 of them in successive years. Eight of these women were then studied more intensively, and two of them in still greater detail. These again illustrated individual variation in quantitative response between the two subjects studied in strict parallel, though the differences here were not so large as between the two subjects intensively studied by Storvick and Hauck.

Fincke and Landquist (1942) studied further the quantitative relations between levels of intake and the resulting concentration levels in the blood plasma of healthy young men and women. To maintain a plasma level of 0.8 mg. per cent required intakes of 0.8 to 1.2 mg. per kgm. of body weight per day, while to maintain saturation required 1.7 to 2.0 mg. of vitamin C per kgm. of body weight per day.

Still further experimental studies of the effects of graded intakes of vitamin C upon the plasma level and urinary excretion have been made by Dodds and MacLeod (1944) with four subjects in 1942 and eight subjects in 1943, all of whom were healthy people. Here again individual physiological variation was found to be an unexpectedly large factor. Daily fluctuations were also relatively large. On the other hand, when experimental periods were long, the average finding for an individual became a reproducible result. Also by successive determinations it can be shown whether at a given intake level the person is losing or gaining. When plasma values are definitely shown to be stabilized in the range characteristic of maximum blood levels, the intake is shown to equal or exceed the amount required to maintain saturation as the term is understood in this connection

In general, higher daily intake results *both* in higher levels of vitamin C concentration in the body and higher levels of output through the kidneys.

At the time this is written (1951–52) there is still considerable dif-

erence of opinion as to the benefit of keeping the body "saturated" ith this vitamin when people have been known to live for relatively ng times, if not indefinitely, at lower concentration levels without ne appearance of specific symptoms of scurvy.

Notwithstanding the fact that an initially healthy man may go nonths with extremely little ascorbic acid before developing distinct mptoms (Crandon, Lund, and Dill, 1940; Lund and Crandon, 941), there is evidence from human experience and animal experi- nentation that *best* results are to be expected only when the ascorbic cid content of food and resulting concentrations in the body are ept not only above but far above scurvy levels.

Even before it had been chemically identified, some investigators ad pointed out the advantage of larger amounts of the antiscorbutic actor than are needed for prevention of frank scurvy. Thus Plimmer rote that in people on scorbutic diets there is a period of poor ealth before definite symptoms of scurvy appear, and that the same ort of poor health may also occur (and sometimes become chronic) n those who habitually take suboptimal amounts of vitamin C ven though they get enough to prevent acute scurvy. Among the esults common in such cases are loss of energy and fleeting pains n the joints and limbs, often mistaken for rheumatism. Also, Hess mphasized the fact that frequently children without showing any istinct scurvy symptoms are irritable, lacking in stamina, and more r less retarded in growth; and can be restored to better growth, igher stamina, and better general health and disposition by the eeding of orange juice or other suitable antiscorbutic food, showing hat the previous food supply had been suboptimal in vitamin C ven though not productive of distinct signs of scurvy.

More recently, the development of much more delicate diagnostic nethods (Kruse, 1943; Kruse and coworkers, 1943) makes it possible or those who specialize in such work to obtain objective criteria of uch borderline states as had been recorded by Hess and by Plimmer. This fact, however, has not yet brought about a complete onsensus of medical opinion because there may be differences in nterpretation of slight variations in the histology of tissues or micro- copic indications of the effects of shortages which may persist as

tissue lesions after the chemical condition of shortage has been corrected.

Vitamin C Requirements in Human Nutrition: The Problem of Adequate and Optimal Intakes

A daily intake of 25 milligrams of vitamin C by normal adults (other than women in pregnancy and lactation who need decidedly more) or 1.0 milligram per 100 Calories of food in family dietaries, might be regarded as a *minimum* sufficing for prevention of the gross signs of scurvy.

In view of the facts now known, it would seem that a satisfactory allowance should provide a wide margin above the bare minimum as a safeguard for the body in its encounters with the varied everyday occurrences which may increase its rate of destruction, and therefore its level of need, of this vitamin.

Double the above amounts, or 50 milligrams for adult maintenance and 2 milligrams per 100 Calories for family dietaries, might be regarded as a *medium* standard.

By again doubling this latter, i.e., by allowing 100 mg. per adult, or 4 mg. per 100 Calories of family food, one may approximate the *presumably optimal* allowance sufficing to keep the body "saturated."

The independent experimental investigations of Dodds and MacLeod, of Fincke, of Hauck, of Ralli, and of Todhunter, with their respective coworkers, agree in showing a daily need of about 1.4 to 1.7 milligrams of vitamin C per kilogram of body weight, or about 100 milligrams for an average adult, to maintain the body in a condition of "vitamin C saturation."

Todhunter and Robbins (1940) have further found, as explained above, that for maintenance of a blood plasma value above 1 mg. of vitamin C per 100 ml., 60 mg. per normal adult per day was required; while a daily intake of 120 mg. was needed to raise the concentration in the blood plasma to 1.4 mg. per 100 ml. Bourquin and coworkers find evidence that more vitamin C is needed when more protein is consumed (Patterson and Bourquin, 1943). This confirms and extends the findings that vitamin C is concerned in the metabolism of certain amino acids.

The Recommended Daily Allowances of the National Research ouncil's Food and Nutrition Board are: Men (average sized, re-rdless of activity), 75 mg.; Women (average sized, regardless of tivity), 70 mg.; pregnancy (latter half), 100 mg.; lactation, 150 g.; Children, under 1 year, 30 mg.; 1–3 years, 35 mg.; 4–6 years,) mg.; 7–9 years, 60 mg.; 10–12 years, 75 mg.; Girls, 13–15 years,) mg.; 16–20 years, 80 mg.; Boys, 13–15 years, 90 mg.; 16–20 years, 00 mg.

These "Recommended Allowances" of the National Research ouncil are so much more liberal than the "standards" of the British uthorities that the Council, in the 1948 edition of its Reprint and ircular Series No. 129, pages 19–20, has offered the following ex-anation:

"Evidence in support of the figures given for vitamin C (ascorbic acid) the Table of Recommended Dietary Allowances has been provided in extensive literature from many research laboratories. The evidence is iefly of the following types: (a) intake levels known to protect against e classical and less severe signs and symptoms of scurvy, for individuals nging in age from infancy to full maturity; (b) studies of intake levels animals and in man, relative to maintenance of specific functions such wound healing, enzyme activity, cellular proliferation, and resistance to ysiological stress; (c) tests of tissue storage at different levels of intake, ith related observations of tissue injury or functional impairment; d) studies of the quantity supplied to infants by human milk when the other's intake of vitamin C (and other nutrients) was at a recognized tisfactory level; and (e) comparative studies in nutrition, including imals that require a vitamin C intake for protection from scurvy guinea pigs and primates) and animals that maintain a 'normal' con-ntration of the vitamin by tissue synthesis.

"Numerous observations have been recorded to indicate the critical vels of intake that are necessary to afford protection from gross signs scurvy, through a period of several months in infants, children, and lults. These levels (approximately one-third to one-fifth of the values ven in the Table [of Recommended Allowances]) obviously are not tisfactory in a long-time appraisal of health and may even result in inical evidence of scurvy when the body is subjected to common forms stress. But it is difficult to state precisely what concentration of scorbic acid in the plasma or blood cells, or what excretion level, should e considered "optimal." The values given in the [National Research

Council's] Table represent a conservative appraisal of all the evidence that is available, but they should not be regarded as "saturation" levels. More generous intakes [may] result in considerably higher concentrations in the tissues.

"The concentration and distribution of ascorbic acid in human tissues, from infancy to old age, is roughly comparable to the concentrations observed in experimental animals under carefully controlled conditions. A progressive depletion can be observed in guinea pigs and primates when there is a deficient intake, but when the intake is at a satisfactory level, the tissue concentrations rise approximately to those observed in animals in which the vitamin is maintained under physiological control (with no intake of vitamin C but with adequate nutrition in other respects). In guinea pigs, it is pertinent to point out that although 0.5 mg. per day will protect young growing animals against the classical signs of scurvy, abnormalities of tooth structure and slowness of wound healing remain evident until a level in the range of 3 mg. per day is reached. Maximum phosphatase activity in wounds during the course of healing is not achieved until the intake is approximately 5 mg. per day. Neither is maximum resistance against injury by bacterial toxins reached until comparable high levels are supplied."

Space permits mention here of only a few of the publications which bear upon the problem of how large a margin above minimal adequacy of vitamin C intake is needed for *best* results including whatever contribution can be made, by this vitamin among other "protective" nutrients, to the body's defenses against injuries from infections and physical strains and to the amelioration of the aging process (or, as McCollum and Simmonds have attractively called it, the conservation of the characteristics of youth).

Goldsmith and Ellinger (1939) found a large proportion of patients to show on testing "a mild vitamin C deficiency" though showing no clinical symptoms. In "saturation" work they found that only when the vitamin C of the blood rose above 1.4 mg. per 100 cc. did urinary excretion increase materially, suggesting that this level ought to be maintained.

Lanman and Ingalls (1937) hold that the body store of vitamin C is of more influence in the healing of surgical wounds in human beings than has hitherto been appreciated, and that liberal intakes of vitamin C should be assured for surgical patients.

ng, Munsun, and Swanson (1940) found that optimal protec- of the teeth from injury by diphtheria toxin required much e vitamin C than is needed for optimal growth and external ap- ance of well-being. And this viewpoint appears to be strongly orted by the critical studies of Kruse and coworkers (1943).

. King emphasizes, another well established observation that ts toward the physiological importance of maintaining fairly intakes of vitamin C is the provision of nature for a high intake scorbic acid by infants. Although fresh cows' milk contains gh ascorbic acid to prevent scurvy, the concentration in mother's is normally about 4 times higher. When the infant receives t milk, tissue concentrations and excretion levels are approxi- ly in the range characteristic of "saturation" status in children adults. Similar tissue concentrations are maintained in guinea- when the food intake is chiefly fresh green food, the animal's ral diet.

achtgens and Werner (1947) find evidence of decidedly in- ed vitamin C requirements during pregnancy.

he recent studies of adult prison inmates, by Sevringhaus and associates (Kyhos, Gordon, Kimble, and Sevringhaus, 1944), vide further evidence in support of the intake levels recom- ded by the National Research Council. Their observations in- ed interesting correlations between well controlled intake levels, ma concentration, and poor or satisfactory gum conditions.

Filkins, in the United States Public Health Service, has recently rved a high incidence of scorbutic gum lesions in areas of the h where there was practically no record of endemic scurvy, but re the intake of vitamin C was known to be low.

fact bearing upon the practicability of high standards of ascorbic consumption is the increased production and consumption of s and fresh vegetables; for it is no longer true that liberal con- ption of fruits and their juices is a luxury out of reach of the . Relatively low prices are practically ensured by the large ard plantings now coming into bearing; and the newer knowl- of nutrition shows fruit to be a good investment up to con- ption levels much higher than yet attained.

Foods as Sources of Vitamin C

Table 46 shows the summary of available data for some typical foods, in terms of the milligrams of ascorbic acid contained in 100 grams of the edible part of each food in the condition in which it is commonly purchased in the market or delivered to the consumer.

Among the foods that enter largely into most American dietaries, the citrus fruits and tomatoes are commonly considered the outstanding sources of vitamin C. They also hold their vitamin C values extraordinarily well in canning and storage.

TABLE 46. VITAMIN C (ASCORBIC ACID) IN CERTAIN FOODS: MILLIGRAMS PER 100 GRAMS OF THE EDIBLE PORTION

FOOD	RANGE WITHIN WHICH FINAL AVERAGE WILL PROBABLY BE FOUND
Apples, medium varieties	5–8
Bananas	6–10
Cabbage, raw	29–52
Cantaloupe	26–35
Grapefruit	37–41
Kale	115–136
Lemons	50–56
Orange (or juice)	49–56
Peas, fresh young green, raw	22–26
Potatoes	14–20
Strawberries	34–60
Tomato (or fresh juice)	22–24
Turnips	25–30
Turnip greens	115–136
Watermelon	6–7

Cantaloupes rank above tomatoes and only moderately below the citrus fruits in antiscorbutic value, and in the times and places of their abundance they may well serve as important sources of vitamin C. Usually, however, cantaloupes are seasonal and expensive while citrus fruits (including canned grapefruit juice) and tomatoes (raw or canned) are now a year-round staple food in most American families.

"Salad greens" (green leaf foods eaten raw or lightly cooked) are a much richer source of vitamin C than generally recognized. Turnip greens, for example, are so rich in vitamin C in their fresh state that if reasonable care to conserve their vitamin value is

rcised in handling and cooking these greens, they should still be important source of vitamin C when used as a cooked vegetable. e same is true of kale.

Cabbage, even of the tight white headed varieties, is rich in tmin C as harvested, but does not hold its value in this respect as l as do tomatoes.

Van Duyne, et al. (1945) found in freshly harvested vegetables in 100 g. of the edible portion: broccoli, 91–172; cabbage, 40–49; roots, 39–160; eggplant, 27–83; mustard greens, 107–142; peas, 41; rhubarb, 56–88; spinach, 43–77; Swiss chard, 25–46.

Mature, resting seeds contain practically no vitamin C. But if *properly sprouted grain or legume* be fed it is found to have considerable antiscorbutic value.

Frozen, concentrated orange juice amply deserves, and is receiving widespread acclaim as an excellent antiscorbutic of high acceptability.

Hamner, Bernstein, and Maynard (1945) have found that the greatest influence on ascorbic acid content of tomatoes was that of variations in light intensity to which the fruits are exposed previous to harvest.

Cooked potatoes, raw apples, and ordinary market milk are typical foods that do not have high concentrations of vitamin C but that are of importance as sources when they enter as largely into the dietary as for other reasons they well may.

In general, the studies of the distribution of vitamin C in plant materials show it to occur most abundantly in the actively functioning and the succulent parts,—in the fresh green leaves, the growing shoots, and the juicy stems, roots, tubers, bulbs, and fruits. It is to be mentioned, however, that in any such simple grouping there may be considerable variations within a group.

All of the commercial fats, sweets, and mill-products of grains are either entirely or practically devoid of vitamin C value.

Muscle tissue contains little vitamin C, but observations upon human scurvy have sometimes indicated that meat, if eaten sufficiently fresh, raw, or "rare," and in large quantities, has an appreciable antiscorbutic value. There is usually a higher concentration in

liver and in glandular organs, including sweetbreads and kidneys.

Eggs, like ordinary meats, seem to contain only traces of vitamin C.

Fresh market milk contains, whether raw or pasteurized, rather more vitamin C than is commonly supposed. Holmes and coworkers (1939), and other recent investigators, have found the loss in pasteurization by modern methods to be about 20 per cent.

Vitamin C is readily changed by oxidation and the greater part of the destruction which takes place upon heating under ordinary conditions is probably of an oxidative nature. Thus Kenny found that the rate of destruction upon heating, which had previously been studied quantitatively by LaMer, Campbell, and Sherman can be reduced about two thirds by rendering the conditions of the heating experiment very rigidly anaerobic; and that the more rapid destruction of vitamin C in cabbage juice than in tomato juice, when both are at the same temperature and hydrogen-ion activity, may be correlated with the higher oxidation potential of the former medium.

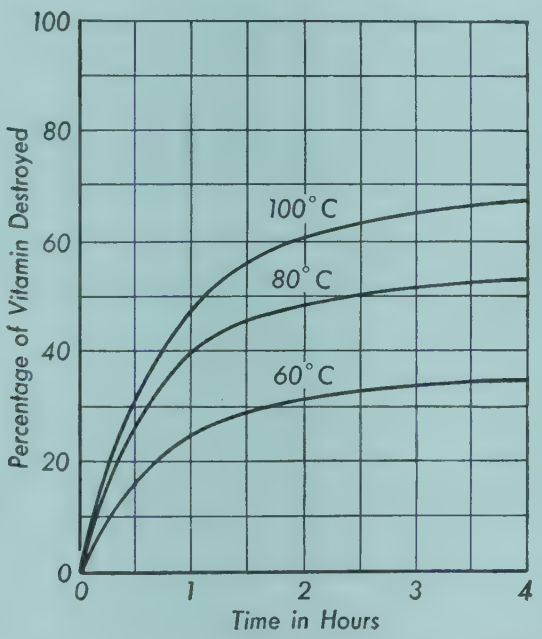
Hence most of the destruction of vitamin C which occurs in such heating as may be applied to foods in household or industrial operations is in some sense due to oxidation; but it is often as the result of heating that such destruction becomes measurable, and the careful quantitative studies which have been made upon the relation of temperature and hydrogen-ion activity to the rate of destruction of vitamin C (as illustrated in Fig. 18) are of permanent scientific and practical significance, whether we explain the chemical mechanism of the destructive process as wholly an oxidation or as somewhat more complex.

With other conditions uniform, a difference in hydrogen-ion activity (pH; "reaction") may be expected to have a marked influence upon the stability of vitamin C.

In tomato juice at its natural acidity of pH 4.2 to 4.3, only 50 per cent of the vitamin C was destroyed on heating for 1 hour at 100°C.; but when the medium was brought to pH 9 before heating, the destruction was increased to 65 per cent. And even in a refrigerator at 10°C. the instability of the vitamin C at pH 9 was such that within

days the destruction just noted had increased to about 95 per cent. Fenton, Tressler, Camp, and King (1938) found that carrots boiled or 15 minutes lost nearly 40 per cent of their vitamin C; but only 1 per cent was actually destroyed while the other 29 per cent was mply leached out of the carrots into the cooking water. When ooked by steaming, carrots retained about 86 per cent of their vita-in C.

To the frequent question whether "adding a little soda in cooking estroys the vitamin," the best answer would seem to be: Not neces-



G. 18. Curves representing the rates of destruction, at different temperatures, the vitamin C of tomato juice at its natural acidity (pH = 4.3).

arily all of it; but always more of the vitamin C is destroyed when oda is added than when it is not. The more soda added the more itamin destroyed; this is true whether or not the change of pH aused is such as to shift it over the neutral point of pH = 7.

When the rate of destruction of vitamin C in tomato juice of atural acidity (pH 4.2–4.3) was studied at the three temperatures 0°, 80°, and 100°C., it was found (as may be seen from Fig. 18) hat both time and temperature are highly significant factors for onsideration in any problem having to do with the stability of

vitamin C and the extent to which it is likely to be destroyed in the practical handling of food materials.

We should also emphasize the fact that ascorbic acid is a freely soluble, readily diffusible substance so that in addition to what is destroyed in cooking or canning, there may be considerable further loss of vitamin C value if the cooking water or the liquid in the can is thrown away.

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Chapter 18

THIAMINE (VITAMIN B_1)

The discovery of thiamine resulted from work along two path of research: laboratory experimentation in normal nutrition, an medical studies of the disease beriberi.

In 1906 Professor (later Sir) Frederick Gowland Hopkins c Cambridge University reported feeding experiments with rats whic showed the need in normal nutrition of some unidentified organi substance or substances soluble in water and in alcohol. Seekin identification of this factor, Hopkins postponed publication of th details of his experiments until 1912. Meanwhile, Dr. Casimi Funk, a Polish chemist working in the Lister Institute (London) had postulated the existence of a factor, the lack of which cause beriberi, and coined for it the name *beriberi vitamin(e)*. Also h predicted (as had Hopkins) that other diseases might be found t be due to shortages of analogous substances.

The Finding of the Nutritional Nature of Beriberi

The disease beriberi was long familiar to the Orient before became well known to the Western world; so long and so widely, i fact, that the origin of the name is uncertain. Some trace it to on language according to which it means a great weakness; others t the word for sheep (in a different language) because the first ou ward sign is usually a stiffness of the ankles giving the victim "sheep-like" gait.

In formal terminology it has been called a *multiple peripher neuritis*, a disease of the nerves of both feet. In severe cases it ma result in paralysis of the legs, and sometimes ultimately in hea failure. Sometimes the heart shows injury first.

In 1878–1883, the entire enlisted force of the Japanese navy numbered about 5000 men, and of these 1000 to 2000 each year were at some time on sick-list with beriberi. The enormous morbidity of 20 to 40 per cent from one disease led to an investigation by Takaki, a high medical officer of the Japanese navy, who worked upon the theory that the fault was most likely to be found in the food since climate appeared to be without influence and the sanitary conditions on the Japanese ships were as good as those in the European navies which were not troubled with the disease. A Japanese naval vessel with 276 men on a 9 months' cruise from Japan to New Zealand, Valparaiso, and Honolulu had 169 cases of beriberi, with 25 deaths. On another vessel with a similar crew, sent over the same route, but with a ration in which the rice was decreased, the barley increased, and vegetables, meat, and condensed milk added, only 14 men had beriberi and each of these had failed to eat his full allowance of the new foods. As a consequence of this experiment, Takaki secured the adoption of a new ration for the entire Japanese navy with the result that the number of cases of beriberi soon became practically negligible.

From his government, Takaki received generous recognition for this really great achievement; and yet it failed to accomplish what might reasonably have been expected of it. Opinion continued to be divided, as it had been before, as to whether beriberi were a nutritional or an infectious disease, with the majority of the medical men of the Orient (where the disease continued to be common) inclining to the theory of an infection. Takaki, as we now know, was right in his belief that the disease was essentially nutritional and he was entirely explicit in saying so, but his work did not carry conviction because he offered no adequate theoretical explanation of it. He discussed his improvement of the ration only in terms of protein, which others rightly regarded as unconvincing. It was a time of great activity in the sanitary applications of the then new science of bacteriology. Sanitary improvements had naturally been made in the Japanese navy during the same years in which Takaki was succeeding in getting the ration changed, and the sanitary improvements were generally given credit for the eradication of the

disease because the germ theory appeared adequate even with the germ unidentified, whereas Takaki offered no satisfactory scientific explanation of what he had accomplished.

In 1897, Eijkman, a Dutch physician working in the East Indies, published observations upon "an illness of fowls similar to beriberi." He had noticed that fowls living in the bare yard of a prison hospital and subsisting almost exclusively upon the left-over rice from the prisoners' table, often showed stiffness and weakness of the feet and legs very suggestive of that of his beriberi patients. By systematic experimentation he found that this disease could regularly be induced in fowls by confining them strictly to a polished-rice diet. This is now generally regarded as the first clear-cut case of experimental production of a dietary deficiency disease. At the time, however, Eijkman's presentation and discussion of his findings was so strongly colored by what English investigators afterward called "the pharmacological bias" that the bearing of his work upon normal nutrition was not made clear. Reading Eijkman's early work in the light of later knowledge, one may take it as marking the initiation of our experimental knowledge of dietary deficiency diseases; but it was not until some years later, and until the experiments had been importantly extended by Grijns (1901), that the results of this work were stated in terms of a clearly nutritional concept.

Meanwhile British and American workers in the Orient were also beginning to give systematic attention to the nutritional aspects of beriberi.

For example, when American Army officers took over the administration of the Bilibid prison in Manila they sought to improve the treatment of the prisoners by furnishing them a better commercial grade of rice—plump, pure-white rice of highest market grade instead of the "low-grade" irregularly shaped brownish rice previously purchased. Shortly thereafter, the proportion of cases of beriberi in this prison population began to increase; but for some time this was regarded (it is to be remembered that the date was 1900–1901) as an epidemic to be combated by sanitary measures. After several months during which everything possible in the way of sanitation had been done, but without conquering the epidemic of

eriberi, attention was turned to the "nutrition hypothesis." Dietary anges analogous to those which Takaki had made in the Japanese avy were tried; and the beriberi was then brought under con-ol.

At about the same time Fletcher, by experimenting with the diet a hospital for the insane, showed that when 28 ounces of rice were d daily with only small amounts of other food, the use of white olished, or of brown unpolished, rice was alone sufficient to de-rmine the occurrence or non-occurrence of beriberi.

In 1909, convinced that beriberi was related to diet, the United tates Army Medical Commission in the Philippines initiated anges in the rations of the Philippine Scouts and in 1911 Chamber-in was able to announce the eradication of beriberi from these oops by the improvement of their ration. In 1908–1909, when beri-eri was at its worst among the Scouts, the ration consisted essen-ally of 12 ounces of beef, 8 ounces of white flour, 8 ounces of otatoes, and 20 ounces of rice (ordinarily polished). The change the ration, as finally decided upon after some months of experi-entation, consisted in giving, in place of the 20 ounces of polished ce, 16 ounces of unpolished rice and 1.6 ounces of dried beans. xperiments, made largely upon fowls, had shown that while beef as some effect in preventing beriberi, an equal weight of beans, eas, or peanuts is much more efficacious. Chamberlain stated, in ct, that the disease had practically disappeared as the result of dding the beans to the ration, before the substitution of unpolished r polished rice had been completed. He held, "that the consump-on of beans to the daily amount of 1.6 ounces would, unaided, have revented a recurrence of beriberi, but it would obviously be diffi-lt to make sure that all the men ate their share of this article over ng periods, and it is therefore much safer that the largest com-onent of the diet, the rice, should be of the unpolished variety and y itself sufficient to prevent neuritis." Several other investigations ave similar results.

Repeated demonstrations of a close connection between diet and e occurrence or prevention of beriberi naturally gave a great npetus to experiments designed to find, and to identify chemically,

the precise substance which has the property of preventing and curing the neuritis which is the outstanding feature of this disease.

The Search for the Antineuritic Substance

As early as 1902, Hulshoff-Pol showed that the antineuritic property could be demonstrated not only with the effective foods themselves, but also with extracts made from them, and even after clarification of such extracts with basic lead acetate and subsequent removal of the lead.

Fraser and Stanton, working in the Malay States, and Chamberlain, Vedder, and Williams in the Philippines, together showed the antineuritic constituent of foods to be an organic substance more stable in acid than in alkaline solution, probably a nitrogen compound, but not identical with any known amino acid or alkaloid. Funk studied the substance further and named it *vitamin*(*e*). Here the introduction of the name of the substance followed by about 10 years its clear apprehension as a definite chemical concept, and preceded by about 15 years its isolation by Jansen and Donath in 1926, while nearly another decade of research was invested by Williams in working out a method of separating the pure substance in sufficient quantity to permit of the establishment of its molecular structure and full chemical nature.

Thus Williams, at great personal sacrifice of his "spare" time for a quarter century, succeeded in identifying the substance and making it available at phenomenally low cost; and now has the satisfaction of seeing the saving of thousands of human lives through the use of the results of his work.

The Chemical Nature of the Antineuritic Vitamin

Space is not available for a description here of the many contributions to the isolation in crystalline form and the working-out of the chemical nature of this substance. It must suffice to say that larger scale production was made possible by the method of Williams, Waterman, and Keresztesy (1934), and that ample evidence soon

upported, and synthesis confirmed, the structural formula proposed by Williams (Fig. 19).

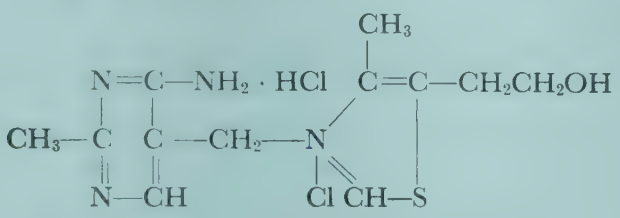

FIG. 19. Thiamine (vitamin B_1) hydrochloride.

It will be seen that the molecule contains a substituted pyrimidine nucleus and a substituted thiazole nucleus linked by a $-CH_2-$ group. This was the first time that the thiazole nucleus had been found in a naturally-occurring substance.

The study of the chemical nature of this vitamin was greatly advanced by Williams' discovery that sulfite at pH 5.0 causes a quantitative cleavage of the molecule into two main fractions. The one which proved to be a thiazole derivative was identified and synthesized by Clarke and Gurin (1935); and Williams and Cline (1936) completed the synthesis and structural identification of the vitamin whose chemical nature Williams had been consistently investigating for about twenty-five years. Williams and Spies (1938) give full evidence for the structure, including an account of the synthetic work.*

Thiamine as the Modern Name

After the molecular structure had been established, the name *thiamine* (thiamine chloride, thiamine chloride hydrochloride) was chosen as the official name of the substance by the Federal Food and Drug Administration, the American Medical Association, and the United States Pharmacopeia. It has also been adopted or endorsed by American scientific societies generally. The term vitamin B_1 continues to be used frequently synonymously with thiamine. The additional synonym, aneurin, is sometimes encountered in European

* For another review of the early chemistry of this substance, see R. R. Williams, *Ergebnisse Vitamin-Hormonforsch.*, **1**, 213–262 (1938).

literature; but its use in this country is definitely discouraged because of the "therapeutically suggestive" character of the word aneurin.

Thiamine in Normal Nutrition

While earlier experiments of Lunin and of Pekelharing may now be seen to have dealt with what have since come to be called vitamins, it was the work of Hopkins which first made the concept effectively clear to most students of normal nutrition. His alcohol extracts of dried milk and of certain dried vegetables were clearly shown to contain some substance or substances essential to growth. We now know that these extracts contained both fat-soluble and water-soluble nutritional factors, and that one of the latter was the substance now named thiamine.

Thus the longest-known functions of thiamine are the promotion of growth and the prevention of polyneuritis.

Thiamine also has an important function in relation to appetite. While all or most nutritional deficiencies tend to result in gradual decline of appetite, thiamine deficiency causes (after a short period in which the body is presumably using up its reserve) a characteristically more abrupt loss of appetite than does any other known vitamin deficiency. Also, thiamine promotes the recovery of appetite in a more prompt and specific way than does any other known nutrient.

And it is not simply that this vitamin makes the food more appetizing: it is an effect of thiamine upon the appetite as a physiological function. For even the feeding of the thiamine *separately* causes the animal to return with appetite to the food which it had refused.

Such observations gave currency to the unqualified statement that this vitamin stimulates appetite. More recently there has been a tendency from two points of view to qualify this statement. From the viewpoint of what is "permissible to claim" it has been proposed to limit the claim for thiamine's action on appetite to a statement that it promotes recovery of an appetite which has been lost because of its lack. And in answer to the question whether stimulation of ap

etite is normally desirable it has been said that thiamine promotes ecovery from a condition of subnormal appetite; and stabilizes ie appetite at the normal level.

It may perhaps be questioned whether the evidence fully supports iese somewhat drastic limitations upon the hitherto accepted mple statement that one of the functions of this vitamin is to romote the appetite. Certainly some scientifically-trained people ave thought they observed increases in already-normal appetites fter taking thiamine. And it seems reasonable to suppose that a positive promotion of appetite is involved in the greater growth hich results from the higher levels of thiamine feeding among ani- hals normal throughout.

Thiamine seems also to function in the maintenance of the normal iotility of the digestive tract. Both L. J. Harris and M. S. Rose rote of a general lack of tone of the gastro-intestinal tract as re- ulting from a shortage of this vitamin. Cowgill, experimenting ith dogs, observed a diminution of vigor in the normal contractions f the stomach as an earlier result of thiamine shortage than the oss of appetite. As the loss of normal motility may extend to the ntestine, the frequently reported constipating effect of dietaries too largely composed of artificially refined food may perhaps be partly ue to paucity of thiamine.

Thiamine functions very importantly in the intermediary metabo- ism. An excellent series of experiments by Peters and his coworkers t Oxford has shown that thiamine, presumably acting mainly in the orm of its pyrophosphate (cocarboxylase), catalyzes the trans- ormation of pyruvic acid and thus helps the carbohydrate metabo- ism through its intermediary stages. Thus thiamine carries the ntermediary metabolism of carbohydrate through an essential step, whether on the way to combustion as fuel or to conversion into fat. This shows thiamine to be a substance of great importance to the working of the active cells in all the bodily systems.

Williams and Spies (1938) emphasized this repeatedly and held hat it affords a scientifically satisfactory explanation of the clinical eports of helpful results from thiamine therapy in a very wide variety of ills.

They also suggested that the abnormal accumulation of pyruvic acid (and perhaps also other intermediates) in the body when thiamine intake is too low may be the cause of failure of appetite and of decline in gastro-intestinal motility and general bodily tone.

To what extent the apparent "toning-up" property of thiamine is limited to "curing the consequences of a previous lack of the same substance," and to what extent it stimulates an already normal appetite and bodily tone, is not entirely clear.

Doubtless this is largely because "B vitamin" has been found to include other things as well as thiamine.

Probably ten or more fairly independent substances may now (1951–2) be considered as generally accepted members of the B group of vitamins: (1) thiamine; (2) riboflavin; (3) niacin (niacinamide); (4) folic acid (pteroylglutamic acid or glutamates); (5) pantothenic acid; (6) pyridoxine (with pyridoxal and pyridoxamine); (7) vitamin B_{12}; (8) choline; (9) biotin; (10) lyxoflavin and from some viewpoints: inositol, para-aminobenzoic acid, and perhaps other substances.

Thiamine in reproduction and lactation. There is abundant evidence that "the vitamin-B complex" is importantly concerned in the nutritional support of successful pregnancy and lactation; but just how this responsibility is shared between thiamine, riboflavin and other factors is still a problem for further research.

Demonstration and Measurement of Thiamine in Foods

Methods Utilizing the Influence of Thiamine upon Growth of Mammalian Species

Rats served as subjects in the work of Hopkins, of Osborne and Mendel, and of McCollum which established the existence of this "new" factor as essential to normal nutrition, and the growth methods for its determination have often made use of this species.

Such quantitative feeding tests can also readily be made to furnish striking demonstrations of both the prevention and cure of nutritional polyneuritis by thiamine and visible evidence of its effect upon growth.

While the effect on the weight curve is usually the best basis for *easurement*, the most *characteristic effects* of shortage of thiamine e failure of appetite and development of polyneuritis. As the ap-tite fails there is also a development of much the same condition is produced by starvation. *Complete* deprivation may even result death without the appearance of the characteristic nerve symp-ms.

Other Methods of Thiamine Assay

Methods based on cure of thiamine-deficient rats or pigeons have een largely used, but more recently thiamine assays are usually ade either by measuring its effect upon the activities of micro-rganisms (microbio-assays) or by *in vitro* methods. Descriptions f analytical methods are not included in this book. Reference may e made to the *U. S. Pharmacopoeia,* and to references at the end f this chapter.

Quantitative Expression of Thiamine Values

Whatever the method employed in measuring thiamine values, ood practice now requires that all quantitative testing include ex-eriments with known amounts of the vitamin side-by-side with ose upon the food or other material under investigation.

When properly conducted and interpreted such quantitative ex-eriments permit statement of the value found in terms of the eight of the actual substance (thiamine, thiamine chloride, or iamine chloride hydrochloride).

Either *the microgram* (*mcg.*) or *the milligram per 100 grams of ood* is usually the most convenient scale of expression.

By resolution of the vitamin committee of the Health Organiza-ion of the League of Nations, the "International Unit of vitamin B_1" as set at three micrograms of the actual substance.

Human Requirements for Thiamine

We have seen that thiamine helps carbohydrate (and doubtless lso the glycerol radicle of fat and the deaminized radicles of some

of the amino acids) through the pyruvic acid stage of metabolism. Hence thiamine deficiency results in a relative accumulation of pyruvic acid in the body; and so, determinations of pyruvic acid in the body's tissues or fluids may be used as evidence as to whether there is a shortage of thiamine in the body at any given time.

Several investigators have made use of this as well as other criteria, including that of "thiamine clearance" (Melnick and Field, 1942) which may be regarded as analogous to the determination of percentage recovery in urine of test doses of ascorbic acid in the studies of vitamin C requirement as reviewed in Chapter 17.

In 1942 Melnick emphasized the objective nature of his thiamine balance studies as evidence of requirements; and considered that from this evidence the thiamine requirement of the human adult could be estimated to be 350 micrograms (millionths of a gram) per 1000 Calories of food. To provide a margin of safety and to cover individual differences he recommended 500 micrograms per 1000 Calories.

Williams, Mason, and Wilder (1943), making use both of objective chemical methods such as Melnick employed and the more subjective neurological tests and observations of preclinical symptoms in subjects living for long periods on thiamine-low diets, concluded that the daily allowance of 0.6 mg. (600 micrograms) per 1000 Calories, as then recommended by the Food and Nutrition Board of the National Research Council, is "none too high."

In a later paper, Melnick (1944) concludes, from a consideration of the influences of various factors as well as individual differences, that the margin of insurance provided by the allowance of 0.6 mg. of thiamine per 1000 Calories of food is to be regarded as desirable.

In the 1948 revised Recommended Allowances, the thiamine is generally set at 0.5 mg. per 1000 Calories; but in pregnancy is increased in greater proportion than the energy allowance, while for men and boys with very high energy needs the thiamine allowance is not increased quite in proportion to the caloric intake.

Here and elsewhere it is to be remembered that the Recommended Allowances of the National Research Council are intended to represent intakes capable of supporting optimal nutritional well-

ing rather than as minimal requirements. Thus we have, in use by e Federal Government at the same time, but for different purposes, an estimated minimum requirement of 1000 mcg., and a commended allowance of 1500 mcg., per day for an average-sized an of such activity as to metabolize 3000 Calories per day.

The Recommended Allowances are: Men (70 Kg.), sedentary, 2 mg.; physically active, 1.5 mg.; doing heavy work, 1.8 mg.; Women (56 Kg.), sedentary, 1.0 mg.; moderately active, 1.2 mg.; ery active, 1.5 mg.; pregnancy, latter half, 1.5 mg.; lactation, 1.5 g.; Children, under 1 year, 0.4 mg.; 1–3 years, 0.6 mg.; 4–6 years, 8 mg.; 7–9 years, 1.0 mg.; 10–12 years, 1.2 mg.; Girls, 13–15 years, 3 mg.; 16–20 years, 1.2 mg.; Boys, 13–15 years, 1.5 mg.; 16–20 ears, 1.7 mg. of thiamine per day.

Thiamine in Typical Foods and in the American Diet

Thiamine is widely distributed in significant amounts in foods of oth plant and animal origin, except such as have been artificially efined or otherwise denatured. Table 47 shows typical foods as llustrations.

ABLE 47. THIAMINE IN EDIBLE PORTION OF SOME TYPICAL OODS: MILLIGRAMS PER 100 GRAMS

FOOD	NUMBER OF STUDIES	NUMBER OF CASES	RANGE WITHIN WHICH THE NORMAL AVERAGE MAY BE EXPECTED TO BE FOUND
Apples	14	32	.02–.04
Asparagus	7	32	.11–.20
Bananas	16	20	.04–.10
Barley (entire)	9	61	.50–.65
Beans, mature	13	37	.40–.70
Beef muscle	20	64	.08–.16
Cabbage	15	38	.04–.08
Oats (oatmeal)	19	56	.54–.82
Oranges or juice	12	19	.07–.09
Tomato	16	17	.06–.10
Wheat (entire)	24	400	.55–.60

Plants synthesize thiamine and while it occurs in the various plant rgans in their natural state, there is a general tendency toward a

higher concentration of thiamine in the mature dry seed than i other parts of the plant.

Unmilled cereal grains are richer in thiamine than the diet as whole needs to be. Thus we saw above that the liberal recommende dietary allowance of the National Research Council is 0.5 m of thiamine per 1000 Calories of food; but whole wheat, whol barley, and oats average about 1.6 mg. per 1000 Calories, an brown rice contains about 0.8 mg. per 1000 Calories. The greate part of this thiamine is in or near the embryo (germ) and oute layers of the grain. Hence such *milled products* as white flour, whi rice, and degerminated corn (maize) meal or hominy, are relative poor in this vitamin,—as well as in others and in mineral element While the quantitative relationships differ with the grains and wit the milling processes used, one may in general expect that the high refined grain product will contain only about one to two tenths much thiamine as the original whole grain,—or not nearly enoug thiamine to provide adequately for the metabolism of the carbo hydrate carried by these foods. Thus diets made up largely of re fined grain products together with refined sugars and fats, tend t be undesirably low in thiamine. *Fortification* of such products (lega istically designated as *enrichment* in the case of breadstuffs, an *restoration* in breakfast cereals) by additions of thiamine with o without other nutrients came to be advocated by many food chem ists and to receive governmental approval and encouragement abou 1940. By 1944 much the larger part of the flour and bread sol in the United States was *enriched*, and some of the States had mad such enrichment compulsory. With breadstuffs thus enriched an a large proportion of breakfast cereal *restored* to approximatel whole grain levels, the thiamine content of the American diet wa (and is) greatly improved. The average thiamine intake of th people of the United States is thus brought to a level about 30 pe cent above the liberal recommendation of the National Researc Council, and the distribution of our national consumption of thia mine is probably more equitable than of most other nutrients be cause in general more bread is consumed by low-income familie But, of course, to ensure this benefit to those who need it most,

s important that enrichment of white flour and bread be made s nearly universal as possible.

Mature dry legumes rank with the whole-grain cereals in thiamine ontent. In this respect as in some others, there is overlapping beween these two groups of foods, while within each group there is onsiderable variation among species and even among cultural arieties.

The other vegetables and the fruits vary still more widely, partly ecause of specific differences among plants, partly because vegeables include different organs of plants, and partly because both ruits and vegetables are marketed sometimes fresh with very high atural water content and sometimes dried.

Among foods of animal origin the thiamine content is relatively ow in milk, higher in eggs and lean meats, and among the meats it s particularly high in pork muscle, while liver and kidney show an mount intermediate between lean pork muscle and the other muscle meats.

Further consideration of the different food groups as sources of his and other vitamins, and of the variations and conservation of hese nutrient factors may be found in Chapters 30 and 31.

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Chapter 19

RIBOFLAVIN

Molecular Constitution and Forms of Occurrence

Riboflavin is the name, formally adopted by the American Medical Association, the Society of Biological Chemists, the American Institute of Nutrition, and the Federal Food and Drug Administration, and now in general use, for the water-soluble yellow substance with greenish fluorescence, which as isolated from milk was called lactoflavin.

Lactoflavin, ovoflavin, and *hepatoflavin* (all recognized as belonging to the group of organic substances known as flavins, and each named according to the natural source from which it was obtained) turned out to be identical, and the same substance became readily available as a synthetic product.

Its molecular structure and corresponding full name are shown in Fig. 20.

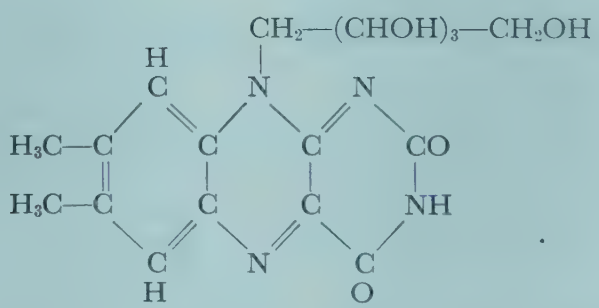

FIG. 20. Riboflavin (6, 7, dimethyl-9-d,l-ribityl-isoalloxazine).

Riboflavin (sometimes free, sometimes in combination with phosphoric acid, often in combination with both phosphoric acid and protein) is very widely distributed in both plant and animal tissues. The so-called respiratory enzyme (Warburg's yellow enzyme) is a combination of riboflavin phosphate with protein. Several such

ombinations are now known. The working-out of their interrelationhips as catalysts of the oxidation processes in the tissues is an active ield of research.

The fact that riboflavin has been identified in such diverse prodcts of life as milk, eggs, many microorganisms, liver, muscles, eyes, eaves, flowers, fruits, and seeds, and in both fresh-water and saltvater algae, raises the presumption that it is almost if not quite niversally involved in the life processes of active cells.

The riboflavin-phosphoric acid-protein combination of typical yelow respiratory tissue enzyme was described by Kekwick and Pederen (1936), on the basis of sedimentation, diffusion, and electrohoresis measurements, as a chemical entity with a molecular weight of the order of about 80,000 and with one flavin group in such a nolecule.

In milk, the riboflavin exists partly as an analogous compound, but also very largely in readily diffusible form, evidently free from combination with any large protein molecule.

Whether riboflavin in food is in the free state, or in combination vith phosphoric acid, or with phosphoric acid and protein, probbly makes little difference to its nutritive value. According to one iew, riboflavin, if free when it reaches the intestine, is combined vith phosphoric acid in the course of its absorption through the intestinal wall.

Hence statements of riboflavin values of foods usually do not distinguish as to whether, or in what proportions, it exists in a free or a combined form.

Nutritional Chemistry of Riboflavin and Its Significance for Well-being

The Journal of the American Medical Association has spoken of riboflavin as "essential to the defense powers of the organism"; and Pinkerton and Bessey (1939) working in the Harvard Department of Pathology have shown that the level of nutritional intake of riboflavin greatly affects the susceptibility of experimental animals to rickettsial disease.

There is abundant evidence that the amount of riboflavin in the food has a large influence upon the level of health and efficiency of people living under ordinary environmental conditions. This is a fact of fundamental importance and should be held clearly in mind when one meets differences of view in regard to the significance of diagnostic signs.

For, while there may be doubt as to the relations of riboflavin to disease, there is no doubt as to its great importance to health, efficiency, vigor, and resistance.

Relation of Level of Riboflavin Intake to Growth, Development, Aging, and Longevity

So direct is the relation of riboflavin to growth that through an important period of the development of our knowledge of riboflavin values of foods, these were determined by means of growth experiments. (See, for examples, Bourquin and Sherman, 1931; Bessey, 1938; Clarke et al., 1940; MacLeod and Taylor, 1944.) That the growth rate (of animals receiving all that they need of other nutrients) is so directly dependent upon the riboflavin intake is readily intelligible when we remember that riboflavin is an essential constituent of all the active body tissues that have been examined for it, and that the riboflavin enzyme is known to be a fundamentally important part of the muscle tissue of which the body is so largely composed.

Perhaps it is because these riboflavin enzymes are so definitely built into the essential substances of muscle tissue, that tissues retain their riboflavin so tenaciously under dietary deprivation. And riboflavin deficiency does not cause so early and rapid a loss of health and body weight as does an equal shortage of thiamine. And when, as often in human experience, there is not a complete deprivation but rather a suboptimal intake of riboflavin, there may be nothing which ordinary medical examination would detect as a deficiency disease. In the young there may be only a slowness of growth and development which the ordinary examiner would consider within the normal range and would either ignore or attribute to inherent physiological variability.

Clearly demonstrated in hundreds of experiments with animals, nd undoubtedly likewise true of our own species, the slowing of rowth which results from suboptimal intake of riboflavin is accompanied by a corresponding retardation of general development. nd if shortage of riboflavin in the food supply continues throughut the period of growth we must expect that the individual's delopment will remain below the level of his inherited or inborn otentiality.

Also it must be expected that such chronic paucity of riboflavin ntake will shorten life, and that to a still greater extent the period f enjoyment of the individual's full adult capacity will be curtailed y the early onset of senility. For photographs of animals showing triking degrees of stunting and premature senility due to chronic hortage of riboflavin, the reader may consult MacLeod and Taylor 1944, pages 302, 307, and 313).

Whether or not one regards riboflavin deficiency as a frequently ccurring disease, it is clear that riboflavin is an important factor in ealth, and in the building of already-normal health to higher evels.

Thus riboflavin is essential to growth, and to normal nutrition at ll ages. When the food is distinctly poor in riboflavin for any conderable length of time, digestive disturbances, nervous depression different from the symmetrical polyneuritis of thiamine deficiency hough both may occur in the same person), general weakness and leterioration of tone, and poor conditions of the eyes and skin are pt to develop; the incidence of infectious disease is likely to be ncreased, vitality diminished, life shortened, and the prime of life urtailed by the unduly early development of the physical manifestaions of old age.

The converse picture is equally true and more attractive.

As compared with the results of a merely adequate or minimaldequate intake of riboflavin, a diet of higher riboflavin value tends o result in better development, higher adult vitality, greater freedom from disease at all ages, somewhat longer life, and (more significantly) a longer "prime of life," i.e., a longer segment of the life cycle between the attainment of adult capacity and the onset f old age.

This extension of the "period of the prime"—a property not of riboflavin alone but of the superior nutritional status or internal environment in which riboflavin is apparently one of the major factors—has been especially emphasized by Simms as important both to the individual and the community because in terms of human affairs it means longer life with a *smaller percentage of years of dependence.*

In controlled experiments extending through two generations of laboratory animals * it has appeared: (1) that riboflavin ranked with calcium and vitamin A as the major factors in the improvement of nutritional wellbeing which resulted from an increase of the proportion of milk in an already-adequate diet; and (2) that in experiments in which riboflavin was the sole significant variable, successive increments of intake resulted in successively increased benefits to nutritional wellbeing, up to levels more than twice that of minimal adequacy. Fuller discussion may be found in Chapters 28, 29, and 33, and in several of the papers listed at the end of this chapter, e.g., Sherman and Campbell (1924, 1930, 1937), Sherman and Ellis (1934, 1939), and Ellis, Zmachinsky, and Sherman (1943). We shall recur in a later chapter to the further consideration of the influence of differences of nutritional intake, above the level of mere adequacy, upon the internal environment of the normal body.

Relation to the Health of the Mouth, Skin, and Eyes

Sebrell and Butler (1938) in their first description of riboflavin deficiency as a human disease for which they coined the name *ariboflavinosis,* emphasized chiefly the symptom which consists in an inflammation of the lips with cracking at the angles (*cheilosis*). Frequently accompanying symptoms are a specific type of glossitis (sore mouth with an abnormally smooth purplish-red tongue) and a dermatitis around the folds of the nostrils. Similarly, dermatitis

* It is probably unnecessary to explain that in this research, as in analogous cases cited elsewhere in this book, the species of experimental animal is chosen for the close resemblance of its nutritional chemistry to that of man, with reference to the nutritional factor or factors under investigation.

as a frequent symptom in the human subjects maintained by Horwitt, et al. (1949) for long periods on a low intake of riboflavin. In riboflavin-deficiency experiments with rats, the dermatitis frequently manifests itself in any of three ways: as "sore nose," or by loss of hair around the eyes, or on the shoulders, the latter two giving rise respectively to the familiar names "spectacle-like" and "saddle-like" dermatitis.

Day and his coworkers showed that riboflavin deficiency causes deterioration not only of the skin but also of the eye (which by origin is a specially developed skin-spot). The correlation of the skin and eye conditions with each other and with cessation of growth in the young, and the experimental conditions under which these three signs of deficiency are governed by riboflavin, were clearly developed in the independent series of papers by Day and by Bessey with their respective coworkers. Their discovery of the sensitiveness of the eye to shortage of riboflavin, made by means of controlled experiments with laboratory animals, has since been strikingly confirmed in the clinical experience of Sydenstricker, Sebrell, Kruse, and others. Several references to this work upon riboflavin deficiency as a human disease will be found below, and the reader may also find additional papers in later literature.

Direct clinical evidence that riboflavin-deficiency is not only a possibility but an actually occurring disease in the United States, is furnished by the work of Sebrell and Butler (1938), Sydenstricker, et al. (1939), Oden, Oden, and Sebrell (1939), Kruse, Sydenstricker, and Sebrell (1940), Sydenstricker, Sebrell, Cleckley, and Kruse (1940), Kruse, et al. (1943), and by several other medical observers. Thus as noted elsewhere, Spies and others have emphasized strongly the frequency of its occurrence in our South; and Aykroyd in a lecture at the U. S. Department of Agriculture in 1943 stated that riboflavin deficiency is the most frequent of the vitamin deficiencies in India, while Braun, et al. (1945) found riboflavin deficiency in 190 of 900 pregnant women examined in Palestine. It has also been reported as of frequent occurrence in several other countries.

Medical reports have emphasized its occurrence both independ-

ently and in conjunction with pellagra. In the latter cases, the much more dramatic symptoms of the pellagra, together with the fact that the two diseases usually both yield promptly to good diet, make it likely that a large proportion of the riboflavin deficiencies are overlooked. For much the same reasons it is probable that the pathology of ariboflavinosis as thus far known constitutes the beginnings rather than a complete picture of the consequences of riboflavin deficiency.

The ocular manifestations reported by Bessey and Wolbach (1939), and by Kruse, Sydenstricker, Sebrell, and Cleckley in January 1940, and described more fully by Sydenstricker, Sebrell, Cleckley, and Kruse in June 1940, are of outstanding interest among the specific symptoms of riboflavin deficiency so far as known at the time of the present writing. The latest of the papers just mentioned (Sydenstricker, Sebrell, Cleckley, and Kruse, 1940) should be read by all who are interested in the symptoms. Slit-lamp examination showed an abnormal vascularity technically designated as "a superficial vascularizing keratitis" with "congestion and proliferation of the limbic plexus" as the earliest and universal symptom in a series of 47 cases, all of which were shown to be riboflavin deficiencies by the readiness with which they were cured by riboflavin alone. Some of these people were hospital patients who had been on "pellagra-producing" diets but were saved from the pellagra symptoms as now understood by means of nicotinic acid as will be explained in the next chapter. Others of the people in this series of 47 were members of the hospital staff who considered themselves well nourished and who came under observation merely because of cracked lips, eyes unduly sensitive to light, dimness of vision, or "eyestrain." While the latter people had chosen their food at will from an apparently adequate food supply, investigation revealed that: "Bad dietary habits with inadequate intake of milk, eggs, and green vegetables were prevalent in the entire group." This investigation adds strong evidence for the view that a large proportion of people need a different emphasis in their choice of food in order to get enough riboflavin to support them at the best level of health of which they are capable.

However, two other facts (more fully discussed elsewhere) are to e remembered in this connection. There is not yet an entire agree- ent of medical opinion as to how far the eye symptoms may be ken as evidence of riboflavin deficiency. And, while unwise choice food may be responsible for much of the riboflavin deficiency at exists, poverty is also undoubtedly often a primary factor.

Relation to Incidence of Infectious Disease

As briefly mentioned above, Bessey and coworkers (Pinkerton and essey, 1939) have definitely shown that the level of intake of ribo- avin has a marked influence upon a certain type of infectious isease in experimental animals; and it is probable that this is like- ise true for man, and for an as yet undetermined number of her infections. As noted above, the American Medical Associa- on based its official "recognition" of riboflavin on the general round of its function in promoting and maintaining the resistance f the tissues. And this view is, of course, strongly supported by the rowing knowledge of the importance of riboflavin as a constituent f tissue enzymes and in the promotion of a high general level of itality and vigor. Thus there is a broad and strong basis for the eneral recognition of liberal riboflavin intake as a factor in re- stance.

Problems of Interrelation of Riboflavin with Other Factors

The occurrence and significance of riboflavin in its different com- inations and its possible interrelations with analogous derivatives f thiamine and of nicotinic acid are subjects of active and com- licated research.

For the purposes of this book, it seems sufficient to call attention the fact that the findings of several investigators show that com- ounds (we do not yet know how many) of thiamine, of riboflavin, f nicotinic acid, and of other B-complex vitamins all function in ie complex enzyme-coenzyme systems which catalyze the oxidation rocess in the body tissues, and that there may be interrelations

in their action. It is partly through studies of the enzymic reactions *in vitro* and partly through very comprehensive feeding experiments under exceptionally accurate laboratory control that one may hope to obtain further light upon such questions as: to what extent do the thiamine, the riboflavin, and the nicotinic acid compounds function independently; to what extent are any of their functions interlinked so that shortage of one impairs the functioning of another; or do any two of them act in parallel to a sufficient extent that an abundance of one may in some degree ameliorate the effect of a shortage of one of the others?

The discovery by Ellis and Zmachinsky (1937 and subsequent experiments) that a high level of riboflavin intake increases the ability to withstand a deprivation of thiamine, may afford an affirmative answer to this last question; or may be interpreted upon the mass-action principle with the hypothesis of a common dissociation product inasmuch as thiamine and riboflavin both contain the pyrimidine nucleus.

Riboflavin has also been found to increase hemeglobin production in dogs which had been rendered anemic by bleeding and then fed a standard basal diet (György, Robscheit-Robbins, and Whipple 1938).

Interesting also is the work of Stannus (1944) identifying riboflavin deficiency with "*pellagra sine pellagra*" and recording his findings as to the sequence in which different body tissues are attacked in riboflavin deficiency: which Stannus prefers to call *hypo-riboflavinosis* rather than ariboflavinosis.

Quantitative Measurement and Expression of Riboflavin Values

According to present knowledge and indications, riboflavin values of foods may be considered as due to the one substance whose chemical nature has been given in the first section of this chapter: and within the limits of normal nutrition at least, the nutritional availability and efficacy of riboflavin is essentially the same whether taken in the free state or in the form or forms of combination in which it largely occurs in plant and animal tissues.

Riboflavin is now so readily available in pure form that all quantitative work on riboflavin values of foods should be controlled, standardized, and interpreted by parallel operations with the pure substance, and the results are best expressed in terms of weight of crystal riboflavin. Milligrams and micrograms (mcg.) of riboflavin per 100 grams of food are the two scales of expression now generally regarded as most convenient, the former being used in this book.

As in the case of thiamine, *in vitro* methods and microbioassays for the determination of riboflavin have been developed and are in general use.

Meanwhile quantitatively controlled feeding methods in which side-by-side experimental animals receive in some cases graded amounts of riboflavin and in other cases graded amounts of the food under investigation, may be used as a "court of ultimate resort."

In drawing the averages for the accompanying table and in commenting the riboflavin data in the Appendix, results obtained *in vitro*, by microbioassay, and by rat-feeding experiments have all been used.

Foods as Sources of Riboflavin

Riboflavin is formed in the growth of green plants whose leaves and stems seem to be richest in riboflavin when in their most succulent stage, notwithstanding the high water content.

Liver and kidneys, milk (whole or skimmed, fresh, canned, or dried), cheese, cream, eggs, lean meats, legumes, and green leaves and buds are typical foods of relatively high riboflavin content. We should not lean too heavily upon comparisons of reported riboflavin contents in foods of different types, because some of the methods of determination are less accurate for some types of food than for others. Hence comparisons *among* meats, for example, are presumably more accurate than comparisons of miscellaneous vegetables *with* meats.

According to current figures, dark meat is much richer in ribo-

flavin than light meat of poultry. Unlike the case of thiamine, riboflavin values of lamb, beef, and pork muscle do not differ strikingly. On the other hand, liver and kidney * contain at least ten times as much riboflavin as the muscle meats, and are among the most concentrated food sources of this factor. It probably is significant that eggs average higher in riboflavin than muscle meats; and cheese (of Cheddar and similar types) higher than eggs. Among the vegetables and fruits, the deep-green leaves and green flower buds (broccoli) are outstanding, containing several times as much riboflavin as do typical fruits and other-than-green vegetables. The fact that sweet corn with its high water content nevertheless has about the same riboflavin content as does mature dry corn illus-

TABLE 48. RIBOFLAVIN IN EDIBLE PORTION OF SOME TYPICAL FOODS: MILLIGRAMS PER 100 GRAMS

	RANGE WITHIN WHICH THE NORMAL AVERAGE MAY BE EXPECTED TO BE FOUND
Almonds	0.5 –0.8
Apple, raw	0.02–0.05
Beans, snap or string	0.10–0.15
Beef muscle	0.15–0.3
Broccoli	0.15–0.3
Cabbage	0.05–0.3
Cheese (Cheddar type)	0.5 –1.0
Eggs	0.3 –0.5
Kale	0.25–0.4
Liver	2.0 –4.0
Milk	0.17–0.25
Peas, fresh green	0.1 –0.3
Pork muscle	0.2 –0.4
Potatoes	0.03–0.05
Sugar	none or negligible
Sweetpotatoes	0.05–0.1
Tomatoes	0.03–0.05
Turnips	0.05–0.10
Wheat, entire	0.10–0.22
Wheat germ	0.6 –0.8

* However, as explained more fully elsewhere, the economics of their production is such that, by the very nature of the case, liver and kidney, even if fully utilized, would not constitute as important a source of riboflavin in the food supply of the whole population, as the muscle meats of which they are a by-product.

rates our general expectation of vegetables in the succulent stage good sources.

The dry, mature, entire grains of barley, corn, oats, and wheat ow very similar amounts of riboflavin, only about one fourth as uch as their thiamine contents.

These whole-grain cereals thus contain significant but not large nounts of riboflavin. Hence the addition of riboflavin in the en- chment of white flour and bread, while bringing the riboflavin ntent to about "whole-grain levels" does not make nearly so large contribution toward meeting the consumer's need in the case of boflavin as in the case of thiamine.

Riboflavin Requirements in Human Nutrition and the Problem of Adequacy of the Food Supply

In its 1948 Allowances, the Food and Nutrition Board of the ational Research Council recommended as follows:

BLE 49. RECOMMENDED DIETARY ALLOWANCES OF BOFLAVIN

an of 70 kg., 1.8 mg.; *Woman* of 56 kg., 1.5 mg.—in both cases regardless activity, as in the parallel allowances of protein.

Woman in second half of pregnancy,	2.5 mg.
Woman in lactation	3.0 mg.
Child under 1 year	0.6 mg.
Child, 1–3 years (12 kg.)	0.9 mg.
Child, 4–6 years (19 kg.)	1.2 mg.
Child, 7–9 years (26 kg.)	1.5 mg.
Child, 10–12 years (35 kg.)	1.8 mg.
Girl, 13–15 years (49 kg.)	2.0 mg.
Girl, 16–20 years (55 kg.)	1.8 mg.
Boy, 13–15 years (49 kg.)	2.0 mg.
Boy, 16–20 years (64 kg.)	2.5 mg.

The discussion accompanying the Recommended Allowances is, part, "Evidence for assessing the desirable allowance of ribo- avin is still rather incomplete. . . . Keys *et al.* (1945) observed physical or functional deterioration in a group of active young en who for nearly six months were supplied with only 0.29 mg. boflavin per 1000 Calories; whereas, Sebrell, Butler, and Wooley 1941) found that in 3 to 8 months a group of sedentary women

receiving 0.21 mg. riboflavin per 1000 Calories developed distinctive physical signs of deficiency, which were responsive to riboflavin therapy. . . . the young men, because of their greater food consumption, received a total of nearly 1 mg. of riboflavin daily whereas the sedentary women ingested but 0.5 mg. per day. . . .

"Williams, *et al.* (1943) found no apparent handicap in four female subjects maintained for 9 months on 13 mcg. of riboflavin per kg. per day. However, neither 13 nor 17 mcg. per kg. prevented a certain degree of depletion of tissue riboflavin. . . .

"It seems likely that very long experimental periods would be required before the consequences of milder deficiency states would become evident."

Thus the situation is that all the experiments with human beings have been for too short periods to show what the allowance would need to be to bring optimal well-being (so far as this factor is concerned) throughout the normal life cycle. The present Recommended Allowances are, in the judgment of the present writer, more than minimal adequate but less than fully optimal.

There are more reasons than the above noted for liberal intakes of riboflavin in human nutrition.

Spies, et al. (1940) have found that riboflavin deficiency "appears to be the commonest of the clinical deficiency diseases among children of the Alabama area studied."

Sydenstricker considered riboflavin deficiency to be perhaps the most frequent of all vitamin deficiencies in the United States; and Aykroyd thought this also true of India.

Street and Cowgill (1939) found that dogs on diet deficient only in riboflavin show no ill effects for a relatively long time (102 to 140 days), then undergo a sudden decline or collapse. Pure riboflavin given by injection or added to the diet prevents the breakdown or cures it if given in time.

It may be taken as strong evidence of the importance of riboflavin to the internal environment, that the body tends to hold its riboflavin content tenaciously, both in cases of shortage of intake of this vitamin, or of pellagra patients (Axelrod, Spies, and Elvehjem, 1941).

Urinary excretion thus becomes a means of judging the adequacy of the body's supply of riboflavin.

Feder, Lewis, and Alden (1944) found "a widespread incidence of riboflavin deficiency," as judged by measurements of urinary excretion of riboflavin, in the Southeastern area of the United States. Stiebeling and Phipard's dietary studies show also a marked prevalence of riboflavin-poor dietaries in the Southeastern states; and in low-cost dietaries of other regions as well.

The wide unexplained variation of riboflavin contents of normal meals found by Kerly (1944), for example, from a low of 0.28 to a high of 0.93 mg. in 14 "entire dinners" served in school canteens, gives added emphasis to the advice that dietary allowances of riboflavin may well be made more liberal than those which are usually accepted as standard.

The per capita consumption of riboflavin in the food of the people of the United States as officially estimated for 1943 and 1944 is practically the same as the Recommended Allowance figured on a per capita basis from the Census data of the age distribution of the population. Hence dietaries of the riboflavin content indicated by the Recommended Allowances are not unduly difficult of attainment, assuming average purchasing power and an up-to-date knowledge of food values. But when decisions as to what foods to buy, and how much of each, are not guided by nutritional knowledge, the higher-income families with their larger per capita purchases of meats, eggs, milk, cheese, cream, and ice cream get more, and the lower-income families get less, than their *pro rata* shares of the riboflavin in the nation's food supply.

One objective of wise nutritional guidance is to raise the lower levels of riboflavin consumption without necessarily lowering the higher levels. In the United States this need not be difficult. It can most readily be brought about by increasing the production and consumption of milk and its products other than butter. This group of foods supplies at present levels about half the riboflavin of the typical American family dietary; and to increase the use of food of this group by perhaps one third in the average lower-income family will give such a family a better-balanced dietary, what-

ever be the shift in the food budget by which the purchase pric is provided.

To some extent the needed shift of emphasis in food productio can be made a matter of national policy, but probably the mo potent incentive to increased milk production will be an informe consumer demand. As is explained in later chapters, an increase proportion of dairy cattle in the farm animal population of th United States will mean both a more efficient use of the Nation and the individual farmer's, food production resources and a bette balanced food supply for the human population.

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Chapter 20

NIACIN (NICOTINIC ACID) AND THE PELLAGRA PROBLEM

The word *pellagra* literally signifies a rough red skin. The victim, called a pellagrin, often has in addition to such a dermatitis, a sore mouth. *Blacktongue* in dogs may be considered an approximate analogue of human pellagra. While the dermatitis and accompanying inflammation of the tongue are the most outwardly characteristic symptoms of the disease, there are often digestive and nervous or psychotic disturbances.

In 1937 Elvehjem showed that blacktongue in dogs can be prevented or (if not too advanced) cured by administration of nicotinic acid, much as beriberi is prevented and cured by thiamine. And within a few months at least four medical research groups had shown the antipellagric value of nicotinic acid and of foods like yeasts which are rich in it. The inappropriateness of the name nicotinic acid for a normal nutrient was recognized and *niacin* was coined as a name which in speaking or writing nutritionally may stand for the *nutrient factor* whether actually present and functioning as nicotinic acid itself or its amide, or the chloride or hydrochloride, or some conjugate.

Nutritional Chemistry of Niacin (Nicotinic Acid)

The normal nutritional functioning of niacin is largely, if not mainly, through the formation of compounds which act as coenzymes.

Thus somewhat analogously to thiamine and riboflavin, niacin serves in the system of tissue enzymes involved in the intermediary and oxidation-reduction metabolism. The complex interrelationships are still in process of being worked out. Unfortunately, niacin alone

oes not enable the typical pellagrin to become a healthy person, r the food supplies which have been responsible for the illness of ost pellagrins are usually deficient in one or more other nutrients ; well. The metabolism of nicotinic acid as indicated by the urinary xcretion of it and its derivatives has been studied by Perlzweig, oldsmith, Sarett, and their coworkers, and by Holman and de ange (1950); and a review of the subject appeared in *Nutrition eviews* for February, 1951. Here it was stated that: "the major rinary excretory products in human subjects are N′-methylnicotinamide and N-methyl-6-pyridone-3-carboxylamide." There is cited a resumably typical experiment in which a subject on a "normal" iet excreted daily 6.3 mg. of N′-methylnicotinamide and 14.2 mg. pyridone; after taking 100 mg. of niacinamide (by mouth), an xcess of 26 mg. of N′-methylnicotinamide and 62 mg. of pyridone ppeared in the urine; while after taking 100 mg. of niacin (i.e., icotinic acid) less of the end-products were recovered, 10 mg. of ′-methylnicotinamide and 26 mg. of pyridone. When 3 gm. of tryp-phane were taken, 2.8 mg. of "extra" N′-methylnicotinamide and 3.6 mg. of "extra" pyridone were excreted—indicating a conversion f this amino acid into niacin or its products.

From such studies it appears that, because of its more complicated ntermediary metabolism, niacin (thus far, at least) yields less clear-t results than do vitamin C and thiamine in studies of the relation f nutritional intake, concentration level in the body, and urinary utput. Nor does there seem to be conclusive evidence as to whether r not it is advantageous to maintain bodily saturation with niacin abitually.

Niacin need not be supplied entirely preformed if certain pre-ursor substances are available in sufficient quantity, for the human ody (and the microbiological population which it harbors), as well s many other organisms, has considerable ability to form niacin rom precursors. Krehl and his coworkers have demonstrated the ransformation of tryptophane to niacin in the rat. It has now been stablished that man, several species of animals, microorganisms, nd the growing corn plant, can accomplish this chemical change. he mechanism of this conversion, the site where it occurs—to what

extent the body tissues accomplish it and to what extent it is due to the activity of intestinal microorganisms—still are not clear, but isotopic studies indicate current progress (see, for example, Review 1950). Methylation may take place and account for some of the recoverable end products.

As explained below, it is still not clear to what extent the human requirement for niacin is met by the niacin formed by intestinal bacteria. It may be largely influenced by the diet.

Kruse (1942, 1942 *b*) studied the influence of *aniacinosis* (niacin deficiency) upon the condition of the tissues, especially of the surface of the tongue. He devised a system of appraising the condition of the tongue in aniacinosis which takes account of the form, intensity, and stage of the departure from normal. The two main categories of form are acute and chronic; then the condition is further classified as to intensity or degree; and finally, as to the stage which the pathological process has reached. Kruse states that in practice it is possible to gauge intensity in three degrees; and stage in four steps if acute, or five if chronic. Also that "if acute and chronic processes are both present,—as most frequently they are,—they can be appraised separately" and then combined in a dual rating of *status*.

In niacin deficiency the tongue and its papillae show vascularity and hypertrophy or proliferation, followed by atrophy of the papillae. Usually the fungiform precede the filiform papillae in undergoing change. Different sites on the tongue may show different stages, the usual order being: tip, anterior and antero-lateral edges, anterior and antero-lateral margins of the dorsum, anterior border of predominantly filiform zone, mid-dorsum. Late in the process, ulcers and erosions may occur on the tongue, usually beginning on the edges. Those desiring a fuller account should read Kruse's original papers.

Niacin or its amide cured all these symptoms, the cures being much quicker in the acute than in the chronic cases. When both acute and chronic pathology were present in the same person, the symptoms of the acute process disappeared first. Kruse emphasizes the same principle here as in analogous cases of other avitaminoses, the chronic cases of long standing can be fully cured only by long-continued treatment. He thinks many patients may have been discharged with only their acute and not their chronic injuries cured.

The specific pathologic changes on the tongue are considered by Kruse (1942 *b*) to be the earliest and most universal and reliable symptoms of niacin deficiency and so of pellagra when thus defined. Kruse also

eaks of this *glossitis* as the earliest discernible tissue manifestation of acin deficiency. He holds that, as the tongue symptoms usually appear rlier than the dermatitis in the development of pellagra, so correspondgly the dermatitis disappears earlier than the glossitis in a cure. And he ggests that too often the pellagra patient is discharged as cured when e skin symptoms are relieved whereas therapy ought to continue until e tongue symptoms also have entirely disappeared which may require year or more.

Prominence of maize in the diet has often been charged, especially European medical literature, with responsibility for pellagra; but is is possibly only true in the same sense that rice eating is related beriberi, that is, too great dependence upon cheap sources of food lories which are but poor sources of the needed vitamin. Possibly ntributing to the effect also is the fact that maize proteins are tably low in tryptophane, which, as mentioned earlier, may serve a precursor of niacin. That cornmeal is not actively pellagraoducing when the rest of the diet is right, is reaffirmed by Kooser nd Blankenhorn (1941) who found that one of two otherwise comarable communities studied has freed itself of pellagra by increased nsumption of milk and eggs, and in lesser degree of lean pork and ickens, with little if any decrease in the consumption of cornheal.

Other Factors Involved in the Pellagra Problem

As Ball (1939), Elvehjem (1939), and S. G. Smith (1940) among thers have emphasized, at least three vitamins of the B group thiamine, riboflavin, and niacin) are collectively concerned in biogical oxidations and any investigation of the role of one of these emands a consideration of the other two.

Independently, Sydenstricker, McLester, Spies, and others emphaze the general experience in pellagrous regions that while nicotnic acid cures the dermatitis and inflamed tongue which are the onditions to which the name pellagra specifically applies, and usuly also alleviates the digestive and nervous disturbances, yet often as noted above) the clinical pellagrin is not thereby rendered a ully healthy person. In fact, Sydenstricker (1941) construed pel-

lagra as a combined deficiency of niacin and riboflavin. Multiple nutritional deficiencies seem, in the actual experience of American people, to be more frequent than deficiencies in niacin alone.

The typical diet of the poor pellagrin contains so little of anything else than milled cereals, sweets, and fats or fat meat that it is all too apt to induce other nutritional deficiencies at the same time with that of niacin.

Physicians dealing clinically with pellagra have repeatedly reported that while niacin cures the pellagra itself, many if not most clinical pellagrins need also thiamine or riboflavin or both. And Spies has reported a group of cases who after receiving all three of these vitamins were still not entirely well, but showed further improvement after receiving pyridoxine (vitamin B_6, see Chapter 22) in addition.

What is most needed in the pellagrous regions is a better food supply. Sebrell gives as the most important foods to add, "milk, liver, lean meats, fish, eggs, tomatoes, green peas, and a variety of green and leafy vegetables such as kale, mustard greens, turnip greens, and collards."

The deficient diet of the pellagrous regions is, he further writes, "caused by the cultivation of a money crop instead of food and forage crops." When even a small fraction of the time and land hitherto devoted to cotton is used for raising vegetables for the family and forage for a family cow, the health, and with it the earning power, of the people should be greatly improved.

The field studies of Sandels and Grady (1932) and of Stiebeling and Munsell (1932) both showed a very clear connection between food habits and pellagra incidence.

It is gratifying to know that the educational work based on such observations as the above, together with opportunity for fuller employment and consequent increased purchasing power have recently very greatly reduced the prevalence of pellagra. Fuller reporting of cases that still occur may tend to obscure the fact in the official statistics, but personal communications from Dr. V. P. Sydenstricker are conclusive that at least in a considerable part of the South there has been a very real reduction in the prevalence of pellagra, at-

butable to improved food habits of the people generally and in-eased purchasing power of previously low-income families.

The Practical Problem of Pellagra Prevention

Largely before the discovery of the relation of niacin to pellagra, e United States Department of Agriculture had made extensive iantitative field studies of the relations of food consumption to llagra incidence and the Public Health Service had experimented tensively with human subjects to ascertain the amounts of different ods that suffice to prevent pellagra. Bringing together the findings these Government researches, it may be concluded that any one the items of Table 50 contains a full day's quota of pellagra-eventive value:

BLE 50. FOOD ITEMS PREVENTIVE OF PELLAGRA

A quart of milk or buttermilk;
A pint of evaporated milk;
One third to one half pound
of dried skimmed milk,
of lean meat,
of canned salmon,
of peanut meal, or
of wheat germ;
One pound of fresh or canned collards,
of kale,
of green peas, or
of turnip greens; or
Two to three pounds of tomatoes, fresh or canned,
or of tomato juice.

Knowing the extreme carefulness of these governmental investiga-ons, particularly in the direct experimentation with human subjects, ne writer believes that the foregoing findings constitute important vidence. That for some foods it does not run parallel with the black-ngue preventive values derived from experiments with dogs may e partly due to pellagra as ordinarily met being less purely a niacin eficiency than is blacktongue and partly to the fact that some foods re better utilized by human beings than by dogs.

With some foods there is, and with some there is not, a good quan-itative correlation between the above direct evidence of pellagra-

preventive value and the current analytical estimates of niacin content. This corresponds with other evidence that present niacin assays both "microbio" and *in vitro*, have more validity for some types of food than for others. These methods appear to be fairly trustworthy for comparisons among meats, or among cereal products, or for control of enrichment in breadstuffs and cereals; but much less so for evaluations of widely different types of food. Another complication lies in the fact that foods may have both direct and indirect niacin values. In addition to the niacin that they actually contain some foods to a large extent—others to only a small extent, if at all—increase the body's niacin intake by favoring the activity of niacin-forming bacteria in the digestive tract. If this is (as it seems to be) a considerable factor in the niacin supply of human nutrition, it must also be subject to large individual variations, which would explain the fact that some patients do not derive the usual amount of pellagra-preventive value from certain foods.

The National Research Council's Recommended Allowances of niacin were set at ten times the corresponding allowances of thiamine for both sexes and all ages. Obviously this was a tentative "first approximation." In 1951–52, however, it is still in force.

As yet, we do not know: (1) what part of the niacin requirement need be contained in the food as eaten, and to what extent a well-balanced diet will be "biologically enriched" in niacin by the bacteria of the digestive tract; or (2) the degree of trustworthiness of present estimates either of the niacin requirements of human nutrition or the niacin contents of some types of food. It therefore appears scientifically sounder not to offer here a table of published niacin values for foods of different types for comparison with each other or with the supposed niacin needs of nutrition.

Grateful acknowledgments for personal communications bearing on different aspects of the foregoing discussion are due to Doctors W. J. Dann, C. A. Elvehjem, W. H. Sebrell, and G. A. Wheeler; but the present writer should bear the blame for any errors or ambiguities in an attempt at interpretation which in any event must be somewhat tentative at this time.

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Chapter 21

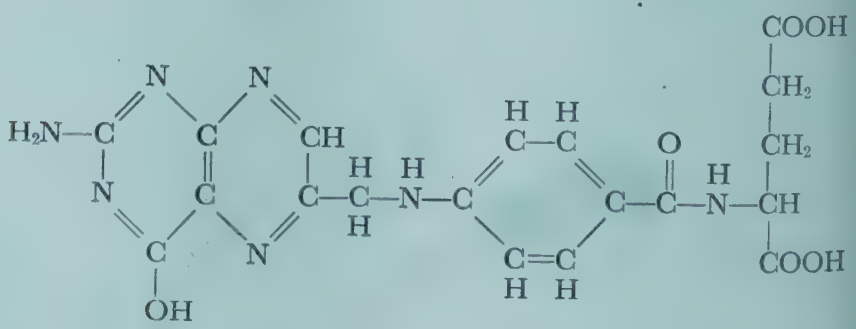
FOLIC ACID, VITAMIN B_{12}, AND CITROVORUM FACTOR

Three substances (nutrient factors) are here made the subje matter of a single chapter because our knowledge of them has largely developed through the studies of the antipernicious anem effects briefly mentioned in Chapter 15.

Folic acid, first designated as vitamin M. is now chemically ide tified as pteroylglutamic acid (or glutamates); vitamin B_{12} is no held to be the specifically antipernicious anemia substance; and th citrovorum factor, into which folic acid is converted *in vivo* in th animal organism, is a substance required for growth by the bact rium *Leuconostoc citrovorum.*

Vitamin M, Folic Acid (Folacin), Pteroylglutamic Acid

Day, Langston, Darby and coworkers found a previously unknow substance to be needed in the nutrition of the monkey. This the designated *vitamin M.* Other groups of workers used *folic acid* *vitamin* B_c, and still other designations for what finally proved to b essentially the same substance in antianemic activity. Chemically was found to be *pteroylglutamic acid* and its formula was estab lished as



Folic Acid

olic acid in nutrition. In 1948 King reviewed in Reprint 129 of National Research Council Reprint and Circular Series three s of evidence that this substance (folic acid, pteroylglutamic l. vitamin B_c, *L. casei* factor, or vitamin M) is essential to nor- human nutrition:

(1) Numerous widely distributed instances have been found in which viduals who revealed no other basis for functional impairment char- rized by macrocytic anemia and associated tissue changes, responded nptly to folic acid therapy, using either the synthetic or the highly ified natural product (pteroylglutamic acid). The dietary histories of e patients have been characterized, in most cases, by an intake of ls that would provide only very small quantities of folic acid.

(2) The characteristic macrocytic anemias identified in infants, chil- n, adult women, and adult men, most logically interpreted in terms n etiology of folic acid deficiency, have been strikingly similar to ditions established by folic acid deficiencies in controlled animal eriments.

(3) Attempts to deplete volunteer subjects of their tissue reserves of c acid by supplying diets adequate in other essential nutrients but in folic acid have met with varying success. Some investigators have found a characteristic depletion in blood, urinary, or fecal concen- ions of folic acid, but in other instances there has been evidence of a v depletion, such as would be anticipated on the basis of animal ex- iments and clinical observations of individuals in an uncontrolled en- nment. Synthesis of folic acid by microorganisms in the intestinal t apparently accounts for a major part of the irregularity in deple- n experiments. The incidence of clinical deficiencies, however, affords dence that intestinal synthesis should not be relied upon as a source this nutrient.

The quantitative requirement cannot be estimated closely from pub- ed evidence. Doan, et al. observed a hematologic response from a sage of 2 mg. of synthetic folic acid per day; Kurnick observed im- vement after a dosage of 2.5 mg. per day; Spies and Suarez observed nission of sprue after dosages of 2.5 to 5 mg. per day of folic acid; d Moore, et al. observed a response from 2 mg. per day. . . . Com- ative studies with monkeys, chickens, and turkeys, on a caloric basis, vide an indirect basis for estimating a human requirement in the ge of 0.1 to 0.2 mg. per day. Many investigators suggest that estimates requirement should be based upon studies of nutritional macrocytic emias and directly related cases rather than upon protection from rnicious anemia."

Folic acid is active hematologically in macrocytic anemias, but in pernicious anemia neither prevents nor alleviates neurological symptoms. However, in macrocytic anemias of infancy and pregnancy, in nutritional macrocytic anemia, and in sprue it is a highly effective therapeutic agent.

Distribution of folic acid. According to R. J. Williams and coworkers (1950, pp. 23–26) the available data on quantitative distribution of folic acid "are too low by a variable and unknown amount" because when these data were determined (Cheldelin, V. H., et al., Univ. Texas Publ. 4237, pp. 15–36) enzymes capable of freeing folic acid completely from tissues were not known.

More recently, however, Toepfer (U. S. Department of Agriculture), in work more especially directed to the quantitative aspect, has determined the folic acid content of many foods, representative cases of which are included in Table 51 (personal communication).

TABLE 51. TOTAL FOLIC ACID CONTENT OF FOODS [a]

	Approximate averages or representative values: milligrams per kilogram (parts per million)
Almonds	0.45
Apples	0.01
Asparagus (fresh)	0.86
Bananas	0.10
Beans, green	0.23
Lima (fresh)	0.34
wax	0.36
Beef, round, I	0.07
Beef, round, II	0.09
Beef, round, III	0.10
Beets	0.16
Bran	1.0
Brazil nuts	0.05
Broccoli	0.33
Cantaloupe	0.04
Carrots	0.06
Cauliflower	0.18
Celery	0.08
Cheese (Cheddar type)	0.12
Chicken, light meat	0.03
Chicken, dark meat	0.03
Chuck (Texas study)	0.15
Corn, sweet	0.14
Eggs	0.03
Filberts	0.71
Heart	0.03

TABLE 51 (Cont.). TOTAL FOLIC ACID CONTENT OF FOODS [a]

Kale	0.42
Kidney	0.58
Lamb	0.03
Liver	2.94
Milk (butter milk)	0.11
Mustard greens	0.38
Oatmeal	0.31
Oranges	0.05
Peanuts	0.61
Peas, green, fresh	0.25
Peas, dry blackeye	4.9
Pecans	0.34
Potatoes	0.06
Pumpkin	0.04
Tomatoes	0.04
Walnuts	0.78
Wheat, entire, I	0.45
Wheat, entire, II	0.41
Yams	0.19

[a] Toepfer, E. W. 1951 Bureau of Human Nutrition and Home Economics, U. S. Dept. Agriculture.

Vitamin B_{12}

In 1926 Minot and Murphy found that pernicious anemia responded to treatment with whole liver or its extract, and during 1927 to 1930 West and Dakin worked actively on the fractionation of liver extracts, testing the fractions in actual therapeutic use with pernicious anemia patients whenever such patients were available. Other research teams also had worked actively in this same field for several years when, in 1948, Smith and Parker in England, and Rickes, Brink, Koniuszy, Wood, and Folkers in the United States were simultaneously successful in the isolation of a crystalline red substance, containing cobalt and phosphorus, which was fully effective (in right conditions) against pernicious anemia. Technical steps in the isolation and chemical identification have been well summarized by Folkers (1950, *Chemical and Engineering News*, vol. 28, page 1634) and need not be repeated here. However, at the time of writing, the chemical structure has not been fully elucidated. The pure substance, designated vitamin B_{12} in 1948, was found to be so active therapeutically that a single dose of 3 to 6 micrograms was clinically

effective when injected intramuscularly in patients with pernicious anemia. Clinical tests have shown that vitamin B_{12} is fully effective in the treatment of pernicious anemia, arresting central nervous system degeneration as well as restoring normal blood values.

Vitamin B_{12}, extrinsic factor, and intrinsic factor. The complete effectiveness of vitamin B_{12} in pernicious anemia led to intensive study of its place in the postulated theory: extrinsic factor + intrinsic factor → erythrocyte maturation factor (see discussion on anemias, Chapter 15); and the role of extrinsic factor has been assigned to vitamin B_{12}.

Berk, et al. (1948) found that vitamin B_{12} administered orally in amounts effective when injected was ineffective unless accompanied by a source of intrinsic factor, e.g., normal gastric juice, but that in very large amounts were fed, it was effective alone. This suggested the identification of vitamin B_{12} with the extrinsic factor, a theory which has been substantiated by the work of various groups approaching the problem from different angles. Other sources of extrinsic factor, such as beef muscle, have been shown to be effective in proportion to their vitamin B_{12} content.*

The role and identification of intrinsic factor is still, however, an unsolved problem. It has been suggested that the function of intrinsic factor is to facilitate the absorption of vitamin B_{12} in some manner. The finding of a substance in normal gastric juice that combines with vitamin B_{12}, making it unavailable for the growth of microorganisms, has been reported by a number of investigators. Ternberg and Eakin have isolated from gastric juice and from hog mucosa a non-dialyzable, heat-labile material, apoerythein, which combines stoichiometrically with vitamin B_{12}. The resulting complex, erythein, is inactive for microorganisms, releases vitamin B_{12} on heating, and is highly selective in its binding action. Vitamin B_{12} is bound by other substances also, such as the lysozyme of eggwhite, but there is a distinction in the complex formed with apoerythein in that vitamin B_{12} cannot be separated from it by dialysis, a readily means of separating it from other protein substances.

* Morgan, E. H., B. E. Hall, and D. C. Campbell 1949 *Proc. Staff Meetings Mayo Clinic* 24, 594.

A study of the reactions between normal gastric juice and vitamin B_{12} by Wolf, et al. (1950) indicates that although it enters into some form of combination, there is no great change in the vitamin itself, and thus strengthens the theory that the function of the intrinsic factor is to promote absorption of vitamin B_{12}, the extrinsic factor.

The interested reader should consult the comprehensive reviews of the status in 1951 of the whole pernicious anemia problem by Castle and by Ungley. See references at the end of this chapter.

Vitamin B_{12} and growth. Vitamin B_{12} has been found to have growth-promoting activity for some microorganisms, for animals, and in some cases for children.

Wetzel and coworkers (1949) have shown strikingly favorable results from vitamin B_{12} in 5 of a group of 11 children who had been making less than normal growth. Although there were no characteristic or suggestive physical signs in the hair, skin, eyes, mouth, or nervous system prior to giving vitamin B_{12}, the growth response was accompanied by improved vigor and alertness, demonstrating that this vitamin has a far more general nutritional significance than its anti-anemic effect alone.

In contrast to this report, Downing could find no improvement in the growth of premature infants given the vitamin intramuscularly.

The growth-promoting property of the so-called "animal protein factor" has been reported to be due in part to vitamin B_{12}, but other effects of this factor, which appears to be of a multiple nature, are due to substances which await the results of further research for identification.

Apparently vitamin B_{12} occurs in at least two active reversible forms that vary in their effect on the growth of microorganisms, although they are both available in analytical feeding tests. The vitamin is known to be formed by many of the lower plants, including certain bacteria, yeasts, and molds. In view of this, it now becomes quite gratuitously confusing to link it with the misnomer of "animal protein factor." Rather may this latter term be dropped in the interest of clarity, both here and in comparisons of proteins.

Vitamin B_{12}, folic acid, and ascorbic acid. Both vitamin B_{12} and

folic acid have been shown to have their hematological effects enhanced in certain cases of refractory megaloblastic anemia by the administration of ascorbic acid. In a deficiency of ascorbic acid in infants, larger amounts of folic acid are required for the remission of anemia. A lack of response to vitamin B_{12} in megaloblastic anemias has been observed by various workers, success being achieved with folic acid alone. Holly has reported the successful treatment of such anemias by the combined use of ascorbic acid and vitamin B_{12}. Studies of Sealock, Day, Darby, and their associates, have demonstrated an interdependence between folic acid, vitamin B_{12}, and vitamin C in regulating the metabolism of phenylalanine and tyrosine. An interrelationship between these three vitamins has also been studied in chicks by Elvehjem and his coworkers who suggest a function of vitamins B_{12} and C in stimulating folic acid synthesis.

Darby and coworkers have shown that either folic acid or vitamin C will correct the abnormal tyrosine metabolism of the scorbutic guineapig.

Studies by Rodney, et al. with liver tissue of folic acid-deficient rats indicate that only folic acid will correct the metabolic defect *in vitro* but that *in vivo* either folic acid or vitamin C is effective.

Vitamin B_{12} appears to be both the much-sought antipernicious anemia factor and an essential nutrient for several species, probably including man. If it shows its indispensability to man less clearly than to some other species, this is thought to be due to the fact that under favorable normal conditions a part of the human adult's need is provided by the activities of the "friendly" bacteria in the intestinal tract; and also that infants may be born with prenatal stores from their mothers.

Distribution of vitamin B_{12} in foods. Methods of assay for vitamin B_{12} are based on its growth-promoting effect for certain bacteria, for the rat, or the chick. Liberation of the vitamin and its separation from interfering substances, as well as the formulation of a suitable medium, have led to some uncertainties in the results of microbial assay, but with improvements in technique and with corroborative results by the growth of rats treated with iodinated casein it appears certain that vitamin B_{12} is limited in its distribution i

common foods. Scheid and Schweigert report values of 40.9–62.3 mcg. per 100 gm. fresh liver, 14.8–23.1 mcg. per 100 gm. fresh kidney, and from 0.9 to 3.2 mcg. for 100 gm. fresh muscle meat. Recent work indicates that apparent vitamin B_{12} values reported for alfalfa and other plant products may in part be due to some unidentified factor or factors, and that the richest food sources of vitamin B_{12} thus far tested are liver and kidney, with eggs, milk, and muscle meats contributing substantial amounts. Robbins, et al. have suggested that the vitamin B_{12} values they find for root vegetables are due to the absorption of vitamin B_{12} formed by soil bacteria.

Vitamin B_{12}, folic acid, and choline. Liver extract has been found effective in promoting growth and preventing the formation of fatty liver in rats on diets devoid of known methyl donors. Bennett (1945) reported that folic acid was not the active principle, nor could the methionine or choline content of the extracts account for the effect. Following the isolation of vitamin B_{12}, a sparing effect of this vitamin on the choline requirement of chicks and rats was observed by Schaefer, et al. (1949, 1950), Bennett (1951), and others. Jukes, et al. (1950), investigating the effect of vitamin B_{12} on the response of chicks to homocystine, concluded that vitamin B_{12} is involved in the synthesis of methionine from homocystine. This was confirmed by Bennett and coworkers in 1951.

Nutritional Functions of Folic Acid and Vitamin B_{12}

The amounts of folic acid and of vitamin B_{12} needed in normal nutrition show rather large and as yet little-studied individual variation.

Folic acid is known to occur in both free and bound forms, and as tri- and hepta- (as well as mono-) glutamate. Additional forms of folic acid contain an extra carbon atom linked as a formaldehyde or alcohol group. Apparently all of these forms are about equally available in animal and human feeding tests, but they vary widely in quantitative tests with microorganisms.

Both in the case of folic acid and of vitamin B_{12}, the output is more largely through the feces than through the urine, and little is

yet known as to what part is due to the food and what part to bio synthesis in the alimentary tract. (See *Reviews*, 1951, 1951 *b*.)

R. J. Williams and coworkers state that the folic acid intak level of 25 mcg. per 100 grams of food suffices for survival in th chick; while 35 mcg. support normal hemoglobin formation; 45 mcg support normal growth; 55 mcg. support normal feathering; and 5 to 110 mcg. support optimal growth.

It is interesting and very significant to compare the graded re sponses to stepwise enrichment of diet by folic acid, with the result of graded intake levels of ascorbic acid as found by C. G. King an reviewed briefly in Chapter 17.

Register and Sarett studied the urinary excretion of vitamin B_1 folic acid, and citrovorum factor in healthy young men on variou diets, and found little effect of diet. In fasting the vitamin B_{12} ex cretion, but not that of the folic acid or citrovorum factor, was abou double the normal value.

The Citrovorum Factor

The existence of this substance was reported by Sauberlich an Baumann in 1948, who found that *Leuconostoc citrovorum*—a non pathogenic bacterium commonly found in milk and dairy produc —failed to grow in media containing all previously known B vitamin and amino acids unless certain crude materials, such as liver concen trates used in the treatment of pernicious anemia, were include This unidentified substance could be partially replaced by hig levels of folic acid and by high levels of thymidine. Because of i occurrence in liver extracts and the low activity of folic acid, it w at first thought that there must be a metabolic relationship of th substance to vitamin B_{12}, but it was soon established by Sauberli and Baumann (1949) and other investigators that the citrovoru factor (CF) differed from vitamin B_{12} and that the latter had growth-promoting action for *L. citrovorum*. Sauberlich (194 1949 *b*) reported further that concentrates of CF overcame the to icity of aminopterin (an inhibitory analogue of folic acid) for *L. c rovorum*, and that whereas rats on a folic acid-deficient diet excret

very little CF in the urine, administration of folic acid either with the diet or by injection resulted in large increases in the CF content of the urine of both rats and man. These results pointed to conversion of folic acid *in vivo*. Such a conversion in rat liver slices was reported by Nichol and Welch (*Proc. Soc. Exptl. Biol. Med.* 74, 52 (1950)), who also found that addition of ascorbic acid with folic acid greatly favored the conversion.

Investigations of the activity of liver extracts in counteracting the inhibitory action of anti-vitamins related to folic acid (e.g., methylfolic acid) by Bardos, et al., and Bond, et al. suggested a relationship of CF to formylfolic acid, a synthetic compound, which had been reported by Gordon, et al. to be more effective than folic acid in counteracting the inhibitory action of methylfolic acid for the organism *Streptococcus faecalis*. This was confirmed by Shive, et al., who reported the production from formylfolic acid of a mixture of compounds which was highly active in promoting the growth of both *Lactobacillus casei* and *Leuconostoc citrovorum* 8081.

Next came the isolation of a crystalline compound possessing the high activity of this reaction mixture as a barium salt by Brockman, et al., and as the free acid by Flynn and coworkers. Later work by Pohland, Flynn, Jones and Shive (1951) on the synthesis of this acid led them to propose for it the tentative structure of 5-formyl-5, 6, 7, 8-tetrahydropteroylglutamic acid. It is commonly spoken of as folinic acid—SF (synthetic factor). That this synthetic factor is not identical with the natural factor has been established by Keresztesy and Silverman who reported the isolation from horse liver of the crystalline barium salt of the citrovorum factor. Assayed with *L. citrovorum*, this barium salt was found to be approximately twice as active as the synthetic factor.

Studies in higher animals with the synthetic factor have given results quite similar to those obtained in microorganisms. For example, Brockman, et al. (1950) and Broquist, et al. (1950) have reported that in mice 15 to 30 micrograms of SF overcame the toxic effects of 10 mcg. of aminopterin; in contrast, 20 mcg. of folic acid had no effect. Very much higher doses of folic acid—up to 5 mg.—were, however, found to be effective by Erschoff and coworkers

(*Proc. Soc. Exptl. Biol. Med.* **73,** 501 (1950)). Cravens and Snell (*Proc. Soc. Exptl. Biol. Med.* **75,** 43 (1950)) and Kline and Dorfman (*Proc. Soc. Exptl. Biol. Med.* **76,** 203 (1951)) have reported that the factor is more effective than folic acid in overcoming the adverse effects of aminopterin in chicks. Similar results have been obtained with rats by Nichol and Welch (*Proc. Soc. Exptl. Biol. Med.* **74,** 52 (1950)) and with pernicious anemia patients by Ellison and coworkers (*Proc. Soc. Exptl. Biol. Med.* **76,** 366 (1951)).

Both citrovorum factor (as a concentrate) and folinic acid (as the crystalline synthetic material) have been shown to have anti-anemic properties for man. The results following intramuscular injections in pernicious anemia patients are less striking than those obtained with vitamin B_{12} or folic acid, but improvement in the blood picture, return to normal of the bone marrow and improvement in clinical condition, but no change in neurological status, have been reported by several independent groups.

Since CF and folic acid have similar activity for all organisms thus far used for the assay of folic acid, it follows that assays for the folic acid content of natural materials must represent the CF plus folic acid content. What proportion of the total activity is due to each form is not as yet known. Schwarz (1951) has reported that in liver 95 per cent or more appears to be in the form of CF.

All studies thus far indicate that folic acid must be transformed to CF within the living organism before folic acid can carry out the catalytic functions for which it is required. In other words, the citrovorum factor either represents the catalytically active form of folic acid or is more closely related to this form than the folic acid itself.

Much has yet to be done to determine the relationship between synthetic and natural CF, the form in which these factors occur in the enzymes of which they are undoubtedly a part, and the nature of the reactions which these enzymes catalyze. The relationship between the function of these factors and that of vitamin B_{12} also needs to be determined.

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Chapter 22

OTHER WATER-SOLUBLE VITAMINS AND SUBSTANCES OF RELATED INTEREST

In the terminology which the British biochemists introduced several years ago, water-soluble vitamins which came to light through differentiation of the original vitamin B concept were treated as members of the "B group" and distinguished by subscript numerals.

With the increasing number of such substances known, many teachers, writers, and research workers have come to regard the term vitamin as "overworked" and some of the "B-vitamins" have been given names coined to suggest as much of their chemical natures as can be conveyed in words of reasonable length. The outstanding example of this is riboflavin—a substance now recognized as of such nutritional importance that it needs no further name; yet which is given prominent place in the recent monograph entitled *Biochemistry of the B Vitamins* (R. J. Williams and coworkers).

On the principle that usage makes language, and with present usage serving as well as it does, we shall not, in this book, give space to any debate as to whether a given substance "should" be called a vitamin or how many water-soluble vitamins "there are." Moreover, because our knowledge of their nutritional significance is still so tentative, it seems best to give only brief statements about the substances mentioned here, and to list references rather liberally at the end of the chapter for the convenience of such readers as may wish to go further.

Some of the substances of this group are presumably essential in human nutrition, but so widely distributed in foods that they are probably rarely if ever "limiting factors" in actual human experience.

Usually, but not necessarily as a rigid rule, we give much the larger part of our present space to substances generally believed to function in human nutrition.

Pyridoxine (Vitamin B_6)

Vitamin B_6 was the first designation of a then unidentified factor required for growth and for prevention of dermatitis in the rat. Because the dermatitis caused by its lack is, in the case of the rat, especially striking, vitamin B_6 was sometimes also called "the antidermatitis vitamin" and confused with the pellagra-preventive factor. Soon after the latter was identified as nicotinic acid, vitamin B_6 was given the name *pyridoxine* (adopted during the spring of 1940 by the American Institute of Nutrition, the American Society of Biological Chemists, and the Council of Pharmacy and Chemistry of the American Medical Association).

Spies, as we saw, reported indications of a vitamin B_6 deficiency disease occurring as a complication of pellagra; but Elvehjem has designated it as a doubtful factor in human nutrition. Its role in nutrition and its therapeutic possibilities are problems for further research.

The conspicuous relationship of pyridoxine as observed in laboratory experimentation is to the prevention of the acrodynia-like dermatitis in rats. There is some evidence that both vitamin B_6 and a fatty acid or fat-soluble factor are concerned in the prevention of this dermatitis; and the latter factor shows similarities to the essential fatty acid of Burr and Burr and to the fat-soluble antidermatitis factor of Hogan and Richardson.

In another aspect of its functioning, vitamin B_6 occurs in several body enzyme systems involved in the metabolism of proteins and amino acids.

A number of recent studies have suggested that pyridoxine is required by man and that blood levels and urinary excretion rise and fall with intake. A naturally occurring deficiency has not been clearly demonstrated but the therapeutic usefulness of pyridoxine in the nausea of early pregnancy has been associated with a possible insufficiency of this factor in such cases.

Pyridoxamine and *pyridoxal* are distinct but chemically related substances which also have vitamin B_6 activity. There is now a tendency to prefer the term "vitamin B_6" where the group of active sub-

stances is met, and reserve the chemical designation for a singl specific substance.

Pantothenic Acid

In 1933 R. J. Williams coined the name *pantothenic acid* to ind cate universal occurrence of this substance in all organisms whic have been adequately examined. It is probably because of the wid natural distribution of this substance that there is as yet no con sensus as to whether it need be supplied to human beings throug their food, or is formed adequately in our bodies or the micro organisms to which our bodies serve as hosts.

As has been found for several other vitamins, pantothenic acid an essential component of an enzyme system, in this case, of a syste controlling acetylation processes (such as the formation of acety choline) in the body.

Vernon (1950) described and discussed a syndrome of painfu "burning" sensation in the feet, observed in prisoners of war, whic he thought had been frequently observed but "inadequately re ported and referred to as dry beriberi." Vernon follows Glusman i calling the disease *nutritional melalgia* and further characterizes as *a deficiency vascular disease*. Vernon writes, "It may represent pantothenic acid deficiency," and according to him, the work o Gopalan indicates that calcium pantothenate yields significant thera peutic results and "seems to establish a relationship of pantotheni acid" to the symptom of "burning feet."

Gopalan (1946) considers that the deficiency disease described i his paper under the title of "burning feet" has certain well-marke and constant clinical features and is a definite clinical entity. I appears to be common among the poor of South India—decidedl more common than "peripheral neuritis" associated with thiamin deficiency.

"Outbreaks of 'burning feet' have previously been reported amon malnourished populations and have often occurred in jails. The di ease has not, however, hitherto been adequately observed and de scribed and has often been confused with peripheral neuritis."

Signs suggestive of riboflavin deficiency were almost always present in the "burning feet" patients, but Gopalan considers the two diseases distinct.

Definite improvement resulted in all of 10 patients who were given calcium-pantothenate only. This improvement was more striking and rapid than that resulting from the yeast concentrate treatment which, however, was also effective.

The explanation for the close association of signs of riboflavin deficiency with signs of pantothenic acid deficiency may lie in the fact that the functions of these two vitamins in human nutrition are closely interrelated, and possibly in a similarity of their distribution in food. The observation of Spies and his associates (1940) that the injection of pantothenic acid causes a rise in the blood level not only of pantothenic acid but also of riboflavin, and similarly that the injection of riboflavin causes a rise in the blood level not only of riboflavin but also of pantothenic acid, is suggestive of a close interrelationship of the two vitamins.

Biotin

Since 1941 a sulfur-containing substance called biotin has been considered as another of the B-group of vitamins. It first attracted attention as preventing the so-called "egg white injury" and has since been found to be essential in the nutrition of several species of animals. Apparently the substance which causes the injury due to excessive egg white is a protein called *avidin* or *avidalbumin;* which combines with biotin, making it unavailable to the body.

Biotin apparently has important functions in intermediary metabolism. It has been recognized as a coenzyme in the synthesis of aspartic acid, which plays a part in a deaminase system; and in several other processes involving the fixation of carbon dioxide (MacLeod and Lardy, 1950).

For further study see the suggested readings at the end of the chapter.

It is still (1951–52) uncertain whether biotin is apt to be of practical importance in human nutrition.

Choline

While differing from most of the substances which we call vitamins in that its chemical nature and wide natural occurrence were well known many years before its nutritional importance became evident, *choline* is now often considered as a member of the "B-vitamin group."

It was noted by Best that choline prevented the abnormal accumulation of fat otherwise occurring in the liver of depancreatized dogs on certain types of diet; and later this same so-called *lipotropic* activity of choline was demonstrated in normal rats fed purified diets of high fat or cholesterol content. This aspect of choline metabolism has been extensively reviewed by Best (1950).

The diets in some of the early experiments were so distorted as to leave considerable doubt whether choline need be considered a factor in normal nutrition; but with increasingly successful efforts to eliminate choline from the experimental rations, it became possible to produce symptoms of choline deficiency without resort to grossly unbalanced diets. One of these symptoms is the disturbance of fat metabolism already indicated, which manifests itself in the development of so-called fatty livers. But, as Griffith has shown, other tissues may be affected as well. Thus he found that on diets still more rigorously freed of choline than is necessary to induce fatty livers, young rats within a very short time showed "hemorrhagic enlargement and degeneration of the kidneys, a regression of the thymus, and an enlargement of the spleen"; and he suggested that choline is essential for the maintenance of the normal structure of tissues as well as for its lipotropic action.

These observations become very suggestive when it is recalled that choline is a component of some of the phospholipids which, as we have already seen, may have both structural and metabolic roles. The effect of choline in preventing the accumulation of fat in the liver was early interpreted as indicating that it facilitates lecithin synthesis, which, according to one theory, is an important step in fat metabolism. Direct evidence in support of this view was obtained by Perlman and Chaikoff who, using radioactive phos

phorus as a tracer, showed that the rate of turnover of liver phospholipids is markedly accelerated by the administration of choline. In ways not yet altogether clear, choline seems to be interrelated in its action with the sulfur-containing amino acids.

It appears also that choline may serve as a methylating agent in body processes. Thus it was found by du Vigneaud and his associates that homocystine (the next higher homolog of cystine) failed to replace methionine in a diet rigorously freed of choline; but that it became a nutritionally satisfactory substitute for methionine when choline was also supplied. Their suggested explanation was that a methyl group may be transferred from the nitrogen of choline to the sulfur of homocysteine, converting the latter to methionine. The reverse transfer was also demonstrated by the finding, after deuteromethionine (methionine containing deuterium in the methyl groups) had been fed, of a higher proportion of deuterium in the body of rats maintained on a methionine-choline-free basal diet. This observation may afford at least a partial explanation of the suggested interrelationship of choline and the sulfur-containing amino acids.

Experimentally, choline has been found nutritionally essential to all species of animals with which it has been fully tested. The requirement appears to be relatively greater in the young than in the adult, and to be increased in lactation.

Lyxoflavin

G. Emerson and K. Folkers have recently reported identification of what appears to be a new member of the vitamin B complex, *L-lyxoflavin*. This new nutrient, a growth factor for microorganisms and rats, is reported to be identical with riboflavin except that the sugar L-lyxose is present instead of D-ribose. (See *J. Am. Chem. Soc.* **73**, 2398 (1951).)

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Chapter 23

VITAMIN A AND ITS PRECURSORS

Chemical Structures and Interrelationships

Vitamin A itself is a colorless, fat-soluble substance,—or more strictly speaking, a pair of such substances,—occurring in relative abundance in the fats of milk, eggs, livers, and fish. It is found in especially high concentration in fish liver oils; and it now appears that there are two closely related substances, vitamin A_1 (which predominates in mammals and salt water fish) and vitamin A_2 (which predominates in fresh water fish liver oils). It is convenient and scientifically admissible to continue to use the term vitamin A as a collective singular to cover these two substances functioning essentially as if they were one. There is, however, another complication which does require recognition in our mode of speaking.

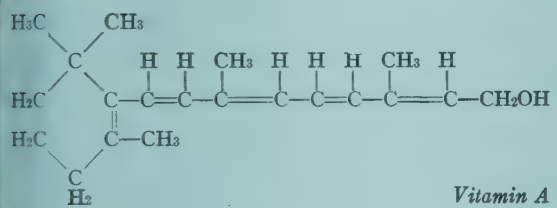
At least four substances (alpha-, beta-, and gamma-carotene and cryptoxanthin) are known to be precursors of vitamin A, and are readily changed to vitamin A in the body. Hence the presence of any of these precursors in a food adds to its vitamin A *value* though not to its vitamin A *content*. The fish liver oils contain vitamin A and practically none of its precursors. Foods generally, however, owe their vitamin A values at least partly to the precursors which they contain.

Green leaves have a high vitamin A value, but no vitamin A content; the value is wholly due to the presence of the precursors. This is believed probably to be the case with the vitamin A values of all plant organs. Animal organs often contain both vitamin A and some unchanged precursor, and this is true of milk and eggs also.

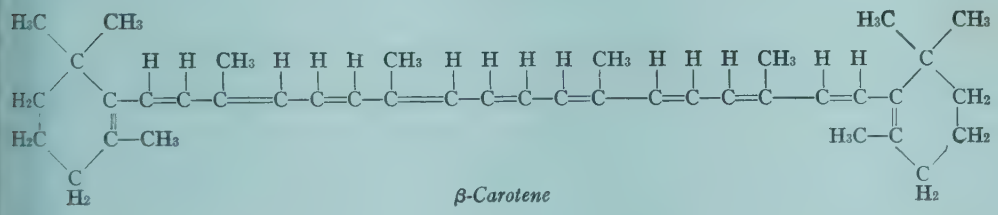
The accepted structural formulae for vitamin A and its two best known precursors are shown in Figure 21.

It will be seen that the structural formula of the beta-carotene molecule, $C_{40}H_{56}$, is symmetrical, with a "double bond" in the center (Fig. 21). By dividing at this point, each half taking on *both* an H and an OH, it is theoretically capable of yielding two molecules of vitamin A, $C_{20}H_{30}O$; while by parallel reactions the other precursors known would each yield only one molecule of vitamin A, corresponding to the half of the precursor molecule here shown on the reader's left, because only this half includes the vitamin A structure.

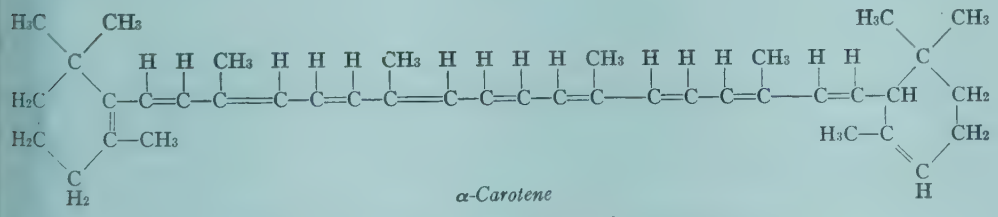
In practice, the body may get considerably less than the theoretical yields of vitamin A from these precursors as they occur in foods. Even so, the vitamin A values of such green leaves as those of kale are high as shown by feeding tests (Sherman and Todhunter, 1934).



Vitamin A



β-Carotene



α-Carotene

FIG. 21. Vitamin A and two of its precursors

The Nutritional Chemistry of Vitamin A

It is of more than historical interest that the existence of what we now call vitamin A was discovered through experiments (made independently and almost simultaneously in 1913 by McCollum

and Davis and by Osborne and Mendel) in which it was found th young experimental animals on diets good in other respects woul continue to grow and thrive or would cease growing and sicke according as the sole fat in their food mixtures was butterfat or lar And further work soon showed that egg fat and cod liver oil resem ble butterfat in this respect while most commercial fats and oils a more like lard. The property, by virtue of which the matern organism concentrates vitamin A in the material (milk or egg provided for the offspring, seems to have a high *survival value* f both mammalian and avian species.

The importance of vitamin A to growth and development is illu strated in the experiments and observations, just mentioned, throug which the existence of this factor was discovered. *Its relations the eyes* are also of great importance.

Vitamin A is directly involved in the chemical changes up which vision depends, and it has been the general view (McCollu et al., 1939, p. 309) that a diminished efficiency of adaptation vision to a changed intensity of light (dysadaptation, hemeralop nightblindness) will be among the earliest observable effects of shortage of vitamin A in an otherwise normal person.

As the vitamin A which thus functions in vision is doubtl brought to the retina by the blood, a lowered concentration of t vitamin in the blood might logically be expected to accompany precede the lowering of visual efficiency; but the reported evide on this point is not entirely clear and consistent.

There is also a difficulty of interpretation as between such che cal evidence as has just been mentioned, on the one hand, and, the other hand, the physical evidence obtainable through biomicr copy. This latter has been especially developed as a diagnos method by Kruse (1941), in whose hands it shows such delica as to indicate that vitamin A deficiencies are considerably m prevalent than the chemical methods indicate. This apparent crepancy may or may not be satisfactorily explained by the fact t what is found by chemical analysis of the blood is something quic responsive to nutritional intake while the Bitot spots and perh other physical signs revealed by the biomicroscope are not

nickly changed by a change of intake. A question still remains as whether a deficiency has been fully cured as soon as the blood hemistry has been brought to normal.

According to the methods and interpretations of biomicroscopy nore vitamin A value should be provided, both for prevention and or complete cure, than has hitherto appeared necessary.

Health Significance of Optimal Intake of Vitamin A Value

Here we meet a somewhat analogous situation to that of ribo-lavin: the relations to health are clearer than the relations to disease. As the normal (rather than the abnormal) chemistry of nutrition is also the primary field of our present study, we give early attention in this chapter to the health relations of the intake levels of vitamin A value.

Relatively early in the history of our knowledge of vitamin A, Dr. F. L. MacLeod found that in sufficiently long-term experiments great differences developed in family life histories of rats fed wheat and milk mixtures according as whole milk or skimmed milk was used (Sherman and MacLeod, 1925). The smaller amount of vitamin A (supplemented, no doubt, by reserves in the bodies of the animals at the beginning of the experiment) was sufficient for normal growth up to nearly average adult size. And the animals presented an excellent appearance during their youth and into adulthood. But this diet did not support successful reproduction, nor did the low-vitamin-A animals attain normal longevity, as did those getting more of this vitamin. In these early experiments, only two levels of vitamin A feeding were used, and these so far apart that the ultimate difference of outcome was too drastic for strictly quantitative measurement.

Dr. E. L. Batchelder (1934) fed animals alike in other respects upon four levels of dietary vitamin A and found that this stepwise increasing of vitamin A intake in the proportions 1, 2, 4, and 8 induced corresponding improvements in nutritional performance and family histories. The gains from extra feedings of vitamin A are summarized in Table 52. The numbers of animals were, how-

TABLE 52. EFFECTS OF DIFFERENT LEVELS OF INTAKE OF VITAMIN A (based on work of Batchelder)

INTAKE	GROWTH AND SIZE	ADULT VITALITY	LENGTH OF LIFE	VITALITY OF YOUNG
At level of 1	Within normal range	Within normal range	Within normal range	Within normal range
Increased from 1 to 2	Slightly increased (within normal range)	Increased (within normal range)	Increased (within normal range)	Distinctly increased
Increased from 2 to 4	Slightly increased (within normal range)	Slightly increased	Slightly increased	Distinctly increased
Increased from 4 to 8	No further change	No directly measurable change	Slightly increased	Distinctly increased

ever, not sufficiently large to permit of smooth quantitative comparisons of results. In later experiments at Columbia similar comparisons were made in larger numbers by Dr. H. L. Campbell and Mrs. H. Y. Trupp.

Here the basal ration was the Columbia Diet A (Laboratory No. 16) on which rat families have thrived throughout 70 to 75 generations so there is no doubt that the diet was adequate to all the nutritional needs of the experimental animals. This basal diet had 3 I.U.* of vitamin A per gram of the dry food mixture, clearly adequate as just explained. When this allowance of vitamin A was doubled the result was a superior record including a longer average life. And when the vitamin A allowance was again doubled, making a fourfold increase over the original (about minimal-adequate) intake, the health record and length of life were still further bettered. Thus vitamin A is one of the outstanding nutrients of which stepwise increased intakes induce correspondingly higher health and longer life up to intake levels about 4 times that of minimal adequacy. There is no doubt that in these experiments the addition of extra vitamin A to a diet which already contained "enough of everything" did positively and constructively build higher health and longer life. And we need have no doubt that this same thing may often occur with people.

The increase in the average length of normal adult life in these experiments was 10.4 per cent with males and 12.1 per cent with females. If one finds doubt about some questions of relation of vitamin A to disease, one can still be confident that it clearly promotes positive health. As we saw in our study of riboflavin, so also is it true with vitamin A that, whatever doubts there are as to its relation to disease, there is no doubt of its important relation to health and longevity; and it is worthy of careful study on this account.

How human beings may best benefit by what has been learned through animal experimentation with different intake levels of vitamin A value, is a problem worthy of careful thought. And, to guide our thinking, we now have much more clearly and firmly established

* I.U. = International Units = U. S. Pharmacopeia Units.

scientific research than existed even a few years ago, or than mo people are probably yet fully aware of.

This animal experimentation upon the responses of the healt and life history has been mainly with the rat because of its clos nutritional similarity to the human. There is no reason to doub and good reason to suppose that, in human as in rat nutrition, be results in long-term experience will require at least 2 to 4 times much vitamin A value as suffices for minimal adequacy. If th minimal-adequate amount is taken as about 3000 I.U. for norm adult maintenance, from 6000 to 12,000 would be a scientifical more logical allowance (than the 5000 I.U. allowance of the Nation Research Council) to provide *both* for individual variations in r quirement and for the maintenance of such a bodily reserve as ha been found to be favorable to higher health and longer life.

To the extent that the vitamin A values of dietaries depend large upon the precursor carotenes rather than on vitamin A itself, an the doubts as to the completeness of availability of these precurso in digestion and assimilation, one will logically prefer to have h intake of vitamin A value nearer the top than the bottom of th 6000–12,000 I.U. zone above suggested.

Also it is scientifically sound in principle to provide liberal intak of a nutrient which seems to be involved in so many different fun tions and in so many major and minor ills as is vitamin A, even some of these involvements are not entirely clear.

The general principle, that nutritional shortages not of sufficie severity to be clearly diagnosed may none-the-less be reasons f dietary improvement, is clearly worthy of especially careful obser ance in the case of vitamin A values. This is a scientifically studie discrimination; not merely an undiscriminating open-handedne with food.

Storage of Vitamin A in the Body May Complicate Interpretation in Research

Vitamin A has a combination of physical and chemical properti which makes it capable of long storage in the body, and up

quantities which are large in relation to the body's daily need. It is to our nutritional advantage that we can thus carry in our bodies such a valuable asset but its importance may be under-valued unless it is kept in mind that the storage factor makes long term experimentation particularly important in relation to vitamin A.

A distinguished pediatrician in the early history of vitamin A tried the experiment of feeding one group of children in an institution skimmed instead of whole milk for 9 months, which he judged would make it a quite thorough test, covering the whole winter season. Finding that this group apparently went through the winter as well as the others, he drew a conclusion which doubtless underestimated the importance of vitamin A for children; because he did not realize the significance of the storage factor, and that a nine months' experiment with children was only the equivalent of about a nine day experiment with rats.

Numerous experiments indicate that the body can store vitamin A at all ages. The very great extent to which bodily storage is influenced by the level of intake in the case of this vitamin is also now well established and will be more fully discussed later in the chapter.

Vitamin A and Vision

From the viewpoint of the chemistry of nutrition, the outstanding relationships of vitamin A to vision are: (1) that vitamin A is essential to the regeneration of visual purple, the bleaching of which in the retina is one of the chemical reactions involved in vision; and (2) as a consequence it follows that the ability to see in a dim light, or to adapt to a changed intensity of light, is very directly related to the abundance and immediate availability of the supply of vitamin A. The mechanism of vision is also two-fold: that of the rods and that of the cones of the (diagrammatic) physical structure of the retina.

Jeans and coworkers (1936), using visual adaptation tests, found seriously frequent evidences of shortage of vitamin A among school children, especially in low-income families. Subsequently much at-

tention has been given to the improvement of instruments for u in such tests, to the critical study of details of technique and inte pretation, and to the extension of this general method both in su veys of populations as to their nutritional status and in laborato research upon the quantitative problems of vitamin A requireme in human nutrition.

Whether measurement of visual adaptation or of the concentr tion of vitamin A in the blood will prove the more valuable criteri is a research question, the answer to which will doubtless depe both upon the development of techniques and upon fuller and mo critical knowledge of the nutritional significance of vitamin It is probable, for instance, that a real shortage of vitamin A the body will inevitably impair the efficiency of visual adaptatio but also that such impairment may sometimes result from oth causes. And because bodily storage may be such a large and i perfectly understood factor, the recent dietary and therapeutic hi tory may not always permit a sound judgment as to whether or what sense a real shortage exists.

Vitamin A and Metaplasia

The relation of vitamin A to mucous membranes is a fact of ve far-reaching significance. It has been found to be important in th eyes, in certain secreting glands, in the respiratory system, the a mentary canal, and the urinary tract; and it is readily conceivab that still other such relationships may remain yet to be di covered.

Mori, Bloch, and Blegvad independently described diseased co ditions of children which in the light of all evidence now availab seem to have been due primarily to shortage of vitamin A. Whi xerophthalmia was the symptom which called attention to the cases, it was noted that there was also a high incidence of respir tory disease which was probably primarily due to the weakening the respiratory system by the same dietary deficiency. Blegva states that the deaths which occurred among the children wh showed the eye trouble were due in most cases to respiratory di

ıse. See also the immediately following section and the readings ıggested at the end of the chapter.

Experimental Deficiency in Laboratory Animals

In experiments with laboratory animals the most readily ob-ervable sign of vitamin A deficiency is usually the development of n ophthalmia (xerophthalmia, keratomalacia, conjunctivitis). This as first recorded by Osborne and Mendel in 1913 and has occurred o regularly (at least in the experience of most observers) as to be egarded as a fairly characteristic result of a lack of vitamin A. But is only one of several results, and frequently not the most im-ortant. For further studies have shown that vitamin A deficiency esults also in an increased incidence: of respiratory disease; of kin, ear, and sinus infections; of inflammations and infections of he alimentary tract; and even of renal calculi (Osborne and Mendel, 1917). Doubtless the underlying cause of the weakness vhich may show itself in so many ways is the histological change vhich Wolbach, Howe, and Church have described as follows: "The pecific effect of the absence of fat-soluble vitamin A in albino ats, guineapigs, and humans is found in epithelial tissues. This ffect is the substitution of stratified keratinizing epithelium for the ıormal epithelium in various parts of the respiratory tract, alimen-ary tract, eyes and paraocular glands, and the genito-urinary tract." ee also Wolbach and Howe (1925), Bessey and Wolbach (1939).

The higher incidence of infection observed among individuals n diets of low vitamin A value may be largely due to the fact that he displacement of normal by squamous epithelial tissue implies ıot only a loss of local secretion but also a loss of the *cilia* which ıormally act with the secretion in cleansing the surfaces of the espiratory tract by expulsion of foreign particles. Plainly the nor-nal surface thus tends to protect the respiratory system both from bacteria and from miscellaneous particles which might cause me-chanical injury and so increase the danger of the establishment of infection by such bacteria as may remain. It has also been sug-gested that the local roughness and stickiness which accompany the

breaking-up of the normal tissue (when it is first being replaced by the squamous) may also increase the danger of lodgment of bacteria which would normally have been expelled.

Thus even our present incomplete knowledge shows more than one way in which shortage of vitamin A may increase the danger of infectious disease. Whether it specifically increases *susceptibility in the technical sense* in which bacteriologists and pathologists are apt to use this term, we do not know. Emphasis tends to be laid upon the characteristic metaplasia of epithelial tissue which many investigators have observed to result from deficiency of vitamin A and which structurally impairs what the *Journal of the American Medical Association* has called "the body's first line of defense." This metaplasia of the epithelium has been observed in many parts of the body and in every species which has been thoroughly studied. It probably underlies the external eye symptoms as well as the other ills above mentioned. In Tilden and Miller's experiments upon monkeys, a shortage of vitamin A resulted in injuries to the digestive tract which were often fatal before the development of a conspicuous degree of ophthalmia.

That the influence of vitamin A, unquestionably important at the "first line of defense" against infections, extends also beyond this is shown by several investigations (among others those of Boynton and Bradford, 1931; of Lassen, in 1932; and of McClung and Winters, 1932), in which infective agents have been *injected* under experimental control, and it has been found that the animals more liberally supplied with vitamin A survived better.

In order to avoid confusion, it should be kept in mind that there are wide differences in attitude among those presumably competent to discuss the relation of vitamin A to infection; and that these differences are to a considerable extent explained by the fact that authorities vary in their modes of approach to the problem. Those who have sought to connect vitamin A with the immunological mechanism as commonly conceived have reported negatively. Those whose approach is somewhat broader but who still concentrate upon the search for clearly direct primary relations, tend to report either negatively, or inconclusively. But on the more comprehensive

(if technically less precise) question, there is much positive evidence that the level of intake of vitamin A does (whether directly or indirectly, primarily or secondarily) influence the frequency, or severity, or duration, of infectious disease—not specifically of any one infection alone, and also not equally of all infections.

Both age and previous feeding may influence the results of a vitamin-A-poor diet.

In a typical series of observations, three fourths of the rats placed upon vitamin-A-free food at the age of about one month (end of infancy) developed the characteristic ophthalmia before death; but it developed in only about one fourth of the rats that were from two to nine months old when subjected to the same dietary deficiency (Sherman and Storms, 1925).

The older rats, however, proved more susceptible than the younger ones to lung infection, the frequency of which, in Columbia experience, like that of Steenbock and other investigators, is increased by diet deficient in vitamin A.

Space is not available here for fuller discussion of the physiology and pathology of vitamin A deficiency. The references at the end of the chapter will serve to put those readers who so desire into touch with the original literature and with review articles on this more medical side of the subject. Also, concise summaries from a more physiological viewpoint than that of the present text may be found in Rose's *Foundations of Nutrition* and in Sherman and Lanford's *Essentials of Nutrition.*

The Storage of Vitamin A in the Body

The combination of properties which, as noted previously, makes vitamin A capable of storage in the body, in quantities sufficient to meet its nutritional needs for relatively long times, is of great importance to nutritional wellbeing and health at all ages, and the clear recognition of the magnitude of this factor in the nutritional economy is highly essential to an adequate appreciation of the true significance of the intake of vitamin A values.

At some times, and in some quarters, there has been failure of

due recognition of the importance of the vitamin A value of th food supply, because of the very fact that bodily storage may pro tect from prompt penalty in periods of subsequent shortage.

Both for interpretation of experiments and for the everyday ap plication of our new knowledge, it is well to keep in mind that th body does not quickly acquire nor quickly lose the full store o vitamin A which it is capable of taking on when habitually given liberal intake. Cammack (Sherman and Cammack, 1926) found th several years ago in experiments with moderately liberal daily in takes, and the same fact seems to have been responsible for som otherwise confusing observations after Wald's administration o massive doses. Conceivably it may also have a bearing upon lack o pronounced correlation between bodily stores of vitamin A an either blood values or light-adaptation data in the work of Stein inger, Roberts, and Brenner (1939).

Diagrams and graphs illustrating the striking differences whic result from different bodily stores of vitamin A resulting from di ferent nutritional intakes have been given elsewhere (Sherman an Boynton, 1925, page 1648; Sherman and Cammack, 1926, pages 7 and 72; Sherman and Lanford, 1951, page 260).

Even at weaning time young animals (doubtless includin babies) may already have a considerable store of vitamin A i the body. The body can also store vitamin A at later ages.

In one investigation (Sherman and Boynton, 1925) laborator animals of known nutritional history were killed and dissected an graded weighed amounts of their tissues were fed as sole source of vitamin A to young experimental animals. In this way it wa shown directly that there is an actual storage of vitamin A in cer tain tissues, and it became possible to study quantitatively the dis tribution of the stored vitamin in the body and also to determin directly the extent to which the vitamin A content of the body i influenced by the intake of this vitamin in the food. If adipos tissue and skin be ignored, at least nine tenths of the total vitamin in the body of a well nourished adult rat is found in the live Weight for weight, kidney appeared about 40 times, lung more tha 40 times, and liver between 200 and 400 times as rich in this vitami

as the muscle of the same animal. Moderate differences in the vitamin A content of the food, such as are well within the range of variation likely to be encountered in human experience, resulted in large differences in concentration of this vitamin in the liver, and distinct differences in the amount of it found in lung tissue. That the vitamin content of the lung tissue is thus dependent upon the abundance of this vitamin in the food is of special interest because it has also been found that the higher intake of the vitamin is accompanied by a lower incidence of lung disease. Baumann, Riising, and Steenbock (1934) have also studied the body storage as affected by nutritional intake and the distribution in the body. They assigned about 95 per cent of the body's vitamin A content to the liver.

In another study of storage of vitamin A in the body (Sherman and Cammack, 1926) the placing of animals upon diet devoid of vitamin A was preceded by the feeding for different lengths of time of a diet bountifully supplied with the vitamin. This resulted in very great prolongation of life upon the vitamin-A-free food, showing that large surpluses of vitamin A can be stored under these conditions also. The maximum storage of vitamin A in rats of different ages was closely approached by feeding a ration containing 4 per cent of cod liver oil, which supplied about 80 times as much vitamin A as appeared necessary for adequate nutrition. However, the store does not increase in arithmetical proportion to the richness of the diet in vitamin A, for apparently the animal uses the vitamin provided in the food more freely or less economically as the maximum store is approached.

Since the storage of vitamin A in the body has been shown to be such an important factor, it becomes probable that the supposed lesser need for this vitamin by the adult than by the young is apparent rather than real, and largely attributable to the fact that the adult has had more opportunity to provide himself with a bodily store of this vitamin which then carries him over periods of deficient intake

The Site of Conversion of Carotene to Vitamin A

Following the work of Moore and of Olcott and McCann in 1931, the site of conversion of carotene to vitamin A was generally accepted to be the liver. Conflicting results were reported by other workers but inconsistencies were attributed, at least in part, to species differences.

Recently there has been renewed activity in the investigation of this problem. In 1946, Sexton, Mehl, and Deuel suggested that the intestine might be the site of the conversion, subsequent work in their laboratory showing that in the rat this is actually the case.

At the same time other research groups, including Glover, Goodwin, and Morton; and Thompson and Kon in England, using different techniques and, in addition to the rat, various herbivora and pigs and chicks, also reached the conclusion that the intestinal wall is the site of conversion of carotene to vitamin A, the efficiency with which the change takes place varying in different species.

Absorption and Transport of Vitamin A and Its Precursors

Drummond, Bill, and Palmer (1935) observed that the vitamin A content of the lymph increased after vitamin A or carotene had been ingested, the vitamin A in the lymph being esterified. Popper and Volk (1944) found vitamin A in the lacteals of the rat after oral dosing with vitamin A, absorption taking place from the small intestine, most rapidly between the upper and middle thirds. After oral dosing with carotene, Goodwin, et al. found the vitamin A level in the lymph of herbivora increased, and that no carotene appeared in the blood. Using a fluorescent method, Radice and Herraiz (1947) found in the rat that absorption of vitamin A took place into the lymphatics, most rapidly from the jejunum. Eden and Sellers found marked increase in the vitamin A content of intestinal lymph, especially from the duodenal region, after giving bullocks, sheep, and rats doses of the vitamin, but there was no significant change in the content of either portal or systemic blood, or of lymph from other regions of the body. Further evidence for the lymphatic

route of transport of vitamin A was provided by the work of Goodwin, et al. (1948), in which increased levels of vitamin A were found in the lymph collected by cannula from the intestinal region in goats after feeding either vitamin A or carotene while blood values remained unchanged.

The recent work of Eden and Sellers confirms the earlier observation by Gray, et al. in 1940 that orally ingested vitamin A ester is hydrolyzed in the intestinal tract, the alcohol apparently being the form in which it is absorbed; but it is re-esterified in its passage through the intestinal wall and appears in the lymph almost entirely as the ester. It is also as the ester that it appears in the circulating blood.

Demonstration and Measurement of Vitamin A Values by Feeding Experiments

When a diet lacking vitamin A value but adequate in all other respects is given to a young growing rat, growth continues for a shorter or longer time according to the extent of the stores of this vitamin which the body possesses, after which growth ceases and there usually soon sets in a loss of body weight and a condition of decline in health. About when the body's reserves of vitamin A have been exhausted and growth ceases, a large proportion of the experimental animals begin to develop a characteristic disease of the eye. This usually begins with a swelling of the lids of one or both eyes or with indications that the eye is becoming unduly sensitive to light. Following this there commonly develops an inflamed and catarrhal condition of the conjunctivae, with a bloody or purulent discharge, the lids becoming scabby or sticky. This, with the swelling of the lids and the sensitiveness to light, sometimes results in the eye being found completely closed. If the eye condition is not treated, and the animal continues to live, the cornea may become affected and blindness results. On the other hand the eye condition, if not too far advanced, usually improves quickly upon feeding the animal with any food of adequate vitamin A value.

Thus a demonstration of vitamin A value in a food may be made

by feeding the food to be tested to a young rat which has recently ceased to grow and begun to develop eye trouble from vitamin A deficiency as just described. Under these conditions if the test food has significant vitamin A value it will usually induce resumption of growth and recovery from the eye disease.

And such testing of foods can be made quantitative by standardizing the conditions and measuring the rate of growth induced by the feeding of graded allowances of the test food and of a reference material of accurately known vitamin A value.

The Health Organization of the League of Nations prepared beta-carotene of which 0.6 microgram had, by definition, one International unit of vitamin A value. The U. S. Pharmacopeia unit of vitamin A value is the same; but the Pharmacopeia organization provides a reference material, more convenient for ordinary use than carotene, in the form of a cod liver oil whose value has been measured in comparison with the International Standard.

More recently, the World Health Organization has offered, as a new reference standard, crystalline vitamin A acetate. At present (1951–52) both this and the beta-carotene standard are in use. Description of the method should also include definition of the oil in which the carotene is dissolved.

The same test may be made both qualitative and quantitative by permitting development of the typical sore eye before the feeding of the test food is begun; but if this involves serious illness in the test animal it is apt to render the results more variable, so that for quantitative work the test feeding is usually begun before the test animal becomes seriously ill, while at least one member of each litter or set of test animals is continued on the basal diet alone as a *negative control* in which the typical development of the vitamin A deficiency disease can be observed.

Quantitative measurements of the relative amounts of vitamin A in food can be made by means of sufficiently numerous and carefully controlled feeding experiments with rats which are amply provided with all other nutrients which they require.

The general plan (originally suggested by Drummond and Coward) is to start with normal young rats 21 to 29 days old, feed-

ing them a basal diet * good in all other respects but free from vitamin A. When any surplus store of this vitamin which the body contained at the beginning has been depleted (or sufficiently diluted by the growth of the animal) the body weight ceases to increase and usually remains nearly stationary for a few days. At this time, if not before, each of the animals is placed in a separate cage and kept under controlled conditions. One (or more) of each litter continues to receive the basal ration only, until it dies from lack of vitamin A, thus serving as a negative control. Other animals are fed, in addition to the basal diet, systematically graded amounts of the reference material or of the food which is being tested.

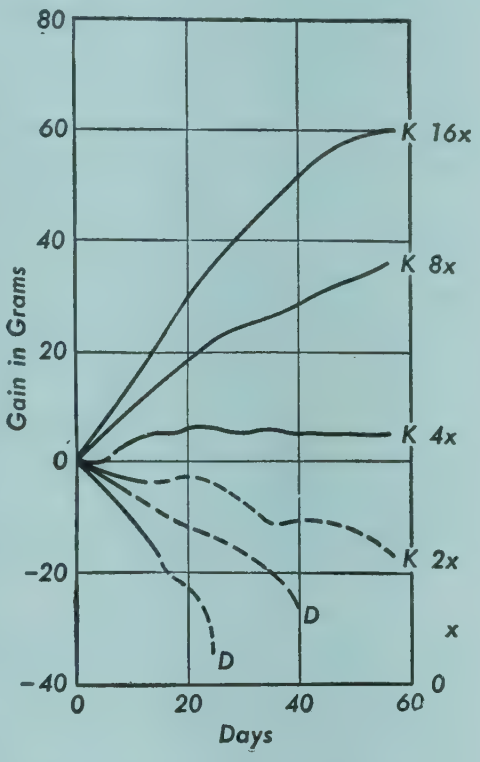


FIG. 22. Average gain-curves of rats fed different amounts of vitamin A after having been depleted of their bodily stores (experiments of Dr. E. L. Batchelder). Relative amounts of vitamin A fed are shown at the ends of the curves. When all animals died before the end of the 8-weeks' period (*K*), the curve is terminated at a point representing the average weight and age at death (*D*). (Courtesy of the *Journal of Biological Chemistry*.)

Natural biological variability makes it probable that the results of individual experiments of this kind will vary more than should the data of chemical experiments conducted *in vitro;* but when sufficient numbers of animals are used and the results are averaged, the average weight curves show a fairly regular gradation according to the amount of vitamin A which the animal actually received.

The accompanying graph (Fig. 22) shows the results obtained by Batchelder in negative controls and in test animals fed at several

* For detailed descriptions of basal diets and techniques of testing, see Coward (1937), and the current *U. S. Pharmacopeia.*

different levels of vitamin A intake (allowance of test food), each curve being the average result obtained with nine test animals. The

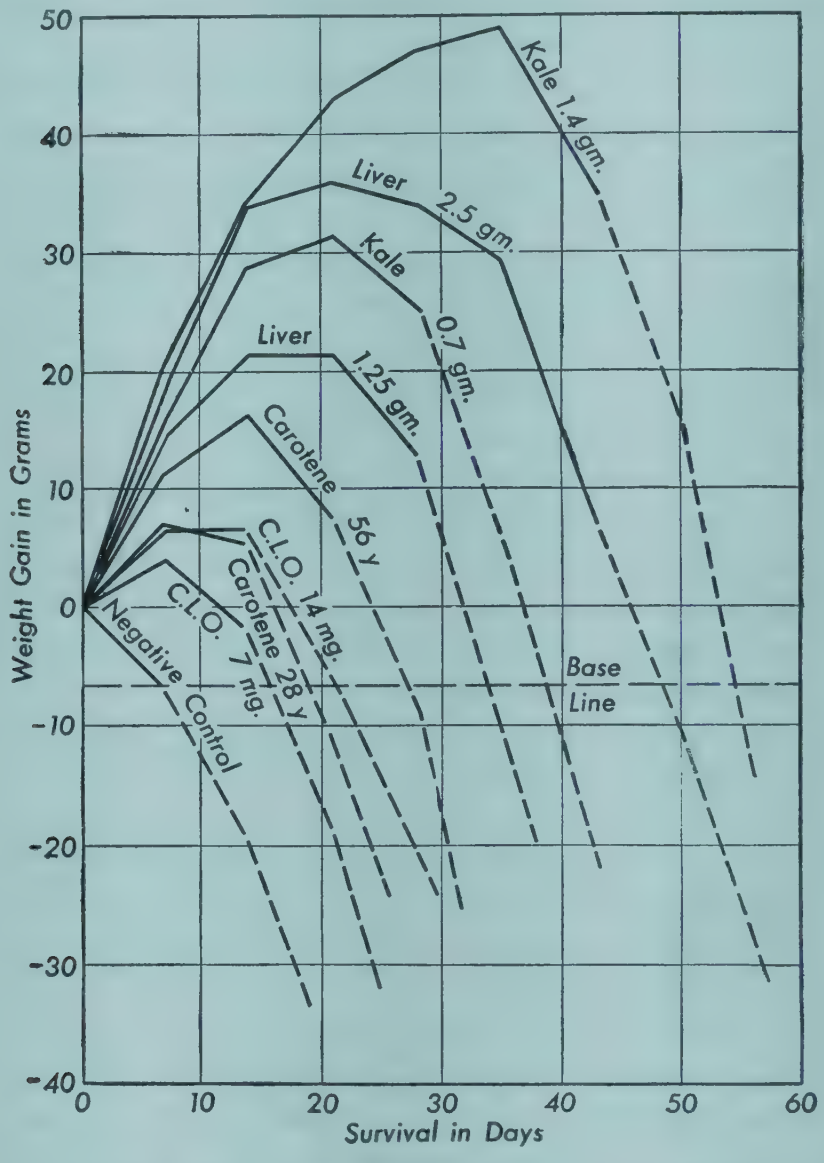


FIG. 23. Average weight curves of rats which had been depleted of bodily vitamin A in the same manner as those of Fig. 22, and to which single feedings of test material were then given. (From experiments by Dr. E. N. Todhunter.)

starting point of these curves is the end of the *depletion period* and beginning of the *test period.*

In the experiments of Todhunter, vitamin A values were deter-

mined by a method of single feedings (Sherman and Todhunter, 1934). The typical results summarized in Fig. 23 are of interest in demonstrating the similarity of weight-curve response to equivalent vitamin A values whether in the form of the vitamin itself (as in cod liver oil) or its precursor (as in the case of carotene or kale) or a mixture of the two; and also in showing the quantitative relationship of weight gain and survival period to the amount of the single feeding of any of these typical sources of vitamin A value. The result of this relationship is that the area, bounded above by the weight curve resulting from a given feeding and below by the negative control curve and the base line, is (under sufficiently standardized conditions) quantitatively proportional to the vitamin A value of the weighed amount of test material used in the single feeding. This makes it possible to measure quantitatively the vitamin A values of materials which are only occasionally available, or of individual samples of fresh foods without subjecting them to the possibility of any decline in vitamin A value due to drying or storage.

Vitamin A Values of Foods

The carotenes which constitute the chief precursors of the vitamin A of normal human nutrition, as well as of ordinary farm animals, are formed in plants. The functional interrelationship of the carotenes and chlorophylls in the nutritional processes of the plant is still a problem of active research. The action of light does not seem to be strictly essential to the formation of carotene, for MacLeod and coworkers have observed an instance of increase of vitamin A value in a root (sweetpotato) on storage, and some small increases have been reported in etiolated seedlings. But, as shown by Drummond and Coward, the pronounced development of vitamin A value in a seedling accompanies the appearance of green color, so that carotene and chlorophyll formation normally go on in a more or less parallel way in young plants and green leaves. The green leaves of food and forage crops and of pastures serve directly and indirectly to supply us with vitamin A values. The high vitamin A values of typical pasture grasses have been accurately

measured by Woods and coworkers (1932, 1935). When we e green leaves, we absorb (with varying degrees of completeness) th carotenes which they contain; and our bodies convert these car tenes (again, with varying degrees of completeness) into vitamin which thus becomes available either for immediate nutrition needs or for storage in the body. And the carotenes of a wider rang of green leaves are brought indirectly into the service of human n trition through farm animals—particularly milch cows and layi hens. The cow and the hen render important services to human n trition by bringing into the readily digestible and assimilable form of milk and eggs a significant share of the nutritive values of leav of coarser quality than we care to eat or which would exceed th quantity that we could readily digest.

In any broad view of the problem of food supply, the outstan ingly important sources of vitamin A value are the green and yello vegetables, eggs, milk, and such products of milk as contain a si nificant proportion of its fat (e.g., butter, cream, ice cream, a whole-milk or "cream" cheeses).* There follows a brief discussio of these and other food sources of vitamin A as illustrated Table 53.

The vitamin A values reported for green leaf vegetables va rather widely, even for the same species. This is doubtless attri utable in part to actual variability of the materials and in part laboratory errors due both to use of methods still in process quantitative development and to differences in the aptitude a experience of the laboratory workers.

The high vitamin A value of the fully green leaf foods, especial kale, is probably much understated by the figure 7540 I.U. for ra kale in *Agriculture Handbook No. 8*. Evidently this does not ta full account of the large numbers of determinations of vitamin values of kale made by Dr. Todhunter in the chemical laboratory Columbia University. Granted that it might be "undue partic larism" to stress any one average from the work of Todhunter, t same criticism is doubtless applicable in at least equal degree

* Liver, with its outstandingly high concentration of vitamin A, is omit from this listing because it necessarily comprises such a small part of the to food supply.

TABLE 53. VITAMIN A VALUES OF TYPICAL FOODS: INTERNATIONAL UNITS PER 100 GRAMS

FOOD	RANGE WITHIN WHICH THE FINAL AVERAGE MAY BE EXPECTED TO BE FOUND
Green leaves such as kale, spinach, turnip greens	(10,000) [a]
Peas, fresh young green	600–1300
Mature seeds generally	nearly negligible
Mill products and breadstuffs	negligible
Sugar and sweets	none or negligible
Fruits: Apples	40–100
Bananas	160–450
Cantaloupes	400–3500
Oranges	50–400
Vegetables: Broccoli	3000–9000
Potatoes	20–50
Sweetpotatoes	1500–7700
Tomatoes	500–1200
Beef muscle	10–50
Milk	160–225
Butter	3300–5000
Eggs	1000–2000

[a] Tentatively assumed average, see text.

some of the figures which did enter into the averages now appearing in the *Handbook*. For instance: chard leaves, 8720; collards, 6870; dandelion greens, 13,650; parsley, 8230; spinach, 9420; turnip greens, 9540 I.U. per 100 grams of the edible portion.

The differences among these figures may very likely be accidental, for kale which appears among the lowest of the fully green leaves in the *Handbook*, is certainly among the highest in fact. The writer has therefore suggested the tentative average figure of 10,000 which is very near the *Handbook* figures for spinach and turnip greens (perhaps the most used greens of the North and South respectively), and as affording a part of the upward revision which is clearly needed in the case of kale.

The evidence now available on the green leaves used as food for man indicates that representative samples contain around 10,000 International Units [a] per 100 grams; but it should be emphasized

[a] As only International Units (which are also the U.S.P. Units) are used in this book, they may be referred to simply as units in this chapter and the one following

that such high values are to be expected only in leaves that are *fully* green. Leaves which are relatively colorless must be expected to be of low vitamin A value. Thus Kramer and coworkers found less than one thirtieth as much vitamin A value in the white inner leaves of head lettuce as in the green outer leaves of the same plants. And the white inner leaves of tight-headed cabbages are of very much less vitamin A value than the green leaves of the loose-leaf cabbages, while the still greener leaves of the closely related kale have much higher vitamin A values.

Quinn, Burtis, and Milner (1927) demonstrated the correlation with greenness in plant tissues other than leaves when they found snap or string beans and green peppers to be of high vitamin A value. Crist and Dye extended this to the case of green *versus* bleached asparagus tips.

The vitamin A values of other parts of plants are in general much lower; but in some cases vary widely with the extent to which carotene is transported to, and stored in, some organs other than the leaf. Thus most roots and tubers are of very low vitamin A value, while the carrot with its high carotene content shows regularly a high vitamin A value, and in sweetpotatoes the value is high but varies widely with the depth of yellow color. But not all yellow plant pigments are precursors of vitamin A; for example, xanthophyll is not. However, F. L. MacLeod has shown that in sweetpotatoes, and M. C. Smith has shown that in squash, the differences in depth of yellow color of the flesh are indicative of corresponding differences in carotene content and thus in vitamin A value.

As already noted, the leaves of forage plants and pasture grasses are of high vitamin A value. The extent to which this value is conserved in hay depends largely upon the curing process. Grains (and therefore our cereals and breadstuffs) are of very low vitamin A value.

As milch cows may consume grains and grasses in quite different proportions, a question arises as to the extent of the resulting variations in the vitamin A values of milk. *In the long run* the nursing mother or the lactating animal is dependent upon her food for all the vitamin A value which she puts into her milk; but the storage

capacity of the body for vitamin A permits a regulatory process by which the vitamin A value of the milk can be maintained during considerable periods of low intake by drawing upon the body stores accumulated in periods in which the intake was high. Thus the vitamin A value of the milk ultimately depends upon, but does not literally vary with and as, the vitamin A value of the food.

The vitamin A values of sea foods originate much as do those of the land. Diatoms, small algae, and other tiny aquatic plants which have thus formed provitamin A may then be eaten by small crustacea, these by fish, and these in turn by larger fish like the cod. Thus the cod, through perhaps two or three or more intermediaries, has obtained its vitamin A from sources ultimately analogous to those which supply the cow.

The U.S.P. standard for cod liver oil calls for not less than 850 units *per gram*, and halibut liver oil is much richer in vitamin A, having, according to McCollum, about 20,000 units per gram, while percomorph oil is still richer.

The Problem of Human Requirements and Adequacy of Food Supplies

The National Research Council's Recommended Daily Allowances for vitamin A values of dietaries, expressed in International Units (I.U.) are as follows: Men (70 Kg.), 5000 I.U.; Women (56 Kg.), 5000 I.U.; pregnancy, latter half, 6000 I.U.; lactation, 8000 I.U.; Children under 1 year, 1500 I.U.; 1–3 years, 2000 I.U.; 4–6 years, 2500 I.U.; 7–9 years, 3500 I.U.; 10–12 years, 4500 I.U.; Girls, 13–15 years, 5000 I.U.; 16–20 years, 5000 I.U.; Boys, 13–15 years, 5000 I.U., 16–20 years, 6000 I.U.

These allowances are materially higher than previous estimates of the amounts needed for the prevention of such signs of deficiency as have been described above, and at the time of adoption early in 1941 were regarded as carrying liberal margins above actual needs. Soon afterward, however, Kruse (1941) by use of a more delicate method, found evidence of a much greater prevalence of shortage of vitamin A than had previously been known or suspected.

Bitot had long ago described the occurrence of elevated spots on the conjunctival membrane of the eye, and these had become known as Bitot spots, but were not generally regarded as significant until Kruse observed repeatedly that they disappeared—although sometimes very slowly—under vitamin A therapy. He therefore considered Bitot spots a sign of vitamin A deficiency (*avitaminosis A*), and proceeded to a biomicroscopic study of the parts of the eye where they are most often seen. This investigation revealed that the typical Bitot spot, visible to "gross" examination, is a relatively advanced sign, the earlier or lesser stages of which can be seen only with the aid of biomicroscopy but are then found to be of more frequent occurrence than the grossly visible spots. Those interested should read Kruse's original (1941) paper for a full account of the microscopic signs which he regards as connected symptoms of tissue changes due to shortage of vitamin A.

If the evidence of this more delicate method be taken as conclusive, it follows that a large proportion of the people previously regarded as amply nourished had actually received too little vitamin A, and that the true human requirement for vitamin A is considerably higher than hitherto supposed. But not all nutritionally-minded physicians yet regard these minute and hitherto unknown or disregarded signs as conclusive evidence of vitamin A deficiency.

At present, therefore, according to one's judgment of these biomicroscopic observations, one may regard the Recommended Allowances of vitamin A value as liberal or as scanty from the point of view of preventing deficiency signs.

And correspondingly one may feel that the average American dietary does or does not carry a fully satisfactory margin of vitamin A value. There seems to be no doubt that the actual vitamin A value of the American food supply has been fully maintained, if not increased, during recent years, due to better conservation of our food (including fishery) resources and increased production of green and yellow vegetables and tomatoes in home gardens. These improvements easily can, and wisely may, be carried further, for medical experience and animal experimentation have clearly shown

that very liberal margins of vitamin A value in the dietary give added benefit in the long run.

Thus, to cite one more view, Mellanby has reported, from his combined hospital experience and animal experimentation, that increased allowances of vitamin A (or precursor) continue to diminish the dangers of infection up to levels of intake four times that required for normal nutrition in absence of exposure.

For people living under everyday conditions and not encountering special exposure to infection, perhaps the best evidence as to long-run desirability of a liberal intake of vitamin A value is that of the above cited full-life and successive-generation experiments with laboratory animals. These experiments showed that successively increased levels of vitamin A intake resulted in successively improved life histories and vigor of offspring, up to diets of about four times the vitamin A value which ordinary criteria would accept as adequate.

By more liberal use of green vegetables the vitamin A value of the American diet can be largely increased at little cost. Thus Gillett and Rice found that families of the same economic grouping in New York City, spending practically the same amount per capita for food in the same market, but according to their own habits and preferences within their economic limitations, were actually consuming four or five times as much vitamin A value per person in some families as in others.

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Chapter 24

THE VITAMINS D AND PREVENTION OF RICKETS

Discovery of the Existence and Nature of Antirachitic Factors

Mellanby found in experiments with puppies that their skeletal development was influenced by some fat-soluble substance or substances in the food. He especially emphasized the prevention of rickets by virtue of an antirachitic property of certain fats.

Almost simultaneously McCollum and coworkers found this true also of rats. Moreover, codliver oil, even after destruction of its vitamin A, still has a specific favorable influence in the prevention of rickets and the promotion of good development of the bones and teeth. The substance thus discovered soon came to be called, interchangeably, *antirachitic vitamin* and *vitamin D*. More strictly we say *the vitamins D*, because it has been found that more than one substance has the property of preventing rickets. These are substances of the *sterol* group which have been mentioned in the section on lipoids in Chapter 3.

As more fully explained by Bills (1939) the term vitamin D (collective singular) is customarily applied to any substance possessing the antirachitic property, which is true of at least ten sterols in some degree, but to an important degree of only two of them: namely, vitamins D_2 and D_3.

Each of these can be produced by the action of ultraviolet light upon its precursor sterol: vitamin D_2 by thus irradiating (activating) ergosterol; and vitamin D_3 by thus activating 7-dehydrocholesterol. This 7-dehydrocholesterol has been shown to occur in the skin and is the chief "animal" precursor of vitamin D_3, just as ergosterol is

the chief "plant" or "vegetable" precursor of vitamin D_2. For th complex structural formulas of these substances, see books on o ganic or biological (physiological) chemistry.

Production of Vitamin D by Irradiation

In 1924 it was discovered by Hess and independently by Steen bock that various foods can be endowed with (or enriched in antirachitic value by irradiation with certain wavelengths of ultra violet light.

Ergosterol, concentrated industrially from yeast, was found to b a good starting material for such activation by irradiation. The pa ticular vitamin D formed by activation of ergosterol is the on known as vitamin D_2. Its commercial forms include calciferol an viosterol. It occurs naturally in fish liver oils, where a mixture o vitamins D_2 and D_3 is present, with the former predominating i some varieties, such as tuna, and the latter predominating in co liver oil.

Vitamin D_3, formed by similar activation of 7-dehydrocholestero is in some cases more effective nutritionally than vitamin D_2, and has been identified with the principal natural vitamin D of cod live oil (and presumably also of milk and eggs). Further activatio studies of Bunker, Harris, and Mosher (1940) have also strengthene the evidence that 7-dehydrocholesterol is the substance which activated, and vitamin D_3 the form which is produced, upon irr diation of the skin.

There is some difference of expert opinion as to whether vita min D_3 is preferable to vitamin D_2 in the problem of the mere pre vention of rickets in infants. In the broader view of child nutritio the fact that vitamin D as obtained in milk and eggs, and in o from fish liver oils, is accompanied by vitamin A is a significan practical reason for preferring such natural sources.

Chicks and rats differ widely from each other in their response to vitamin D_2: for the rat it is a relatively effective antirachiti and the potency of its preparation is measured by tests with ra International and U.S.P. units are therefore rat units. "Rat unit f

rat unit," vitamin D_3 is more effective in the nutrition of the chick than is vitamin D_2.

Bills emphasizes the fact that from a given provitamin only one form of vitamin D is produced by irradiation whatever the wavelength or other conditions. The conditions influence the by-products but the vitamin is always the same for the same precursor. He adds that processes in which electron bombardment is substituted for ultraviolet irradiation produce the same kind of vitamin.

The Prevention of Rickets

According to Park's definition which has been generally accepted, rickets is a condition in which the mineral metabolism is disturbed in such a way that calcification of the growing bones does not take place normally. To guard against confusion, one must keep in mind in reading the literature on rickets that the term has often been given a narrower definition than Park gives it, older definitions turning more upon the histology of the bone tissue while Park's is expressed in terms of calcification in the developing bone—an essentially chemical phenomenon.

The failure of normal calcification in the young bone is not usually to be attributed to any initial fault of the bone itself, for Shipley found that rachitic bones placed in normal blood serum began at once to calcify normally; and Kramer and Howland found in rickets a subnormal concentration of either calcium or phosphorus (or both) in the blood serum. Thus there are different types of rickets which may be classified according to the nature of the chemical deficiency in the blood.

When the calcium content of the serum is normal but its phosphorus (phosphate ion) content is subnormal there results the so-called *low-phosphorus rickets* which corresponds most closely and completely to the classical histological descriptions of the disease, such as those of Schmorl. There is in such cases not only a characteristic pathological histology but also a tendency to overgrowth of the cartilaginous or osteoid tissue at the ends of the long bones and at the rib junctions. Because the retarded calcification prevents the

bones from acquiring normal rigidity at a normal rate, the pressure of the body weight tends further to enlarge the ends of the bones. Together these abnormalities make a typical (and formerly all too familiar) picture recognized in part by the obvious clinical signs of enlarged joints or row of beadlike swellings at the rib junctions and in part by means of the Roentgen ray photograph and the determination of inorganic phosphate in the blood serum; and confirmable at autopsy by histological examination. There has sometimes been a tendency to confine the term rickets or "true rickets" to this low-phosphorus type.

When the phosphorus content of the serum is normal but its calcium content is subnormal there results a similar gross abnormality but a somewhat different histology of the bones. This has sometimes been called a "rickets-like condition" or a "second type of rickets," but is now commonly designated as *low-calcium rickets.* Low-calcium rickets is often accompanied by tetany; and it seems not improbable that in such cases the trouble with the nerves as well as with the bones is attributable (at least in part) to the deficiency (subnormal concentration) of calcium in the blood.

When both calcium and phosphorus are reduced below normal concentration in the blood serum, calcification is retarded, but the structural abnormality of the bone differs from that of the two types described above. The condition constitutes *a third type of rickets* under Park's definition, but because of the differences in clinical and histological appearance it is sometimes called *an osteoporosis* instead.

Unless the mineral supply is deficient, *any of the three types of rickets can be prevented by vitamin D, which acts to restore to normal the concentrations of calcium and phosphate ions in the blood.*

Whence does the vitamin "mobilize" the calcium or phosphate or both? The chief answer seems to be by increasing the *net* absorption of calcium and phosphorus; more being absorbed or less excreted, or both.

This, however, may not be quite the whole story of the action of vitamin D in the prevention of rickets.

Sometimes the calcium or phosphorus mobilized through the

blood stream and thus made available to the developing ends of the bones seems to come from stores already in the tissues.

There are also some indications that a local factor, affecting, as Hess expressed it, the "anchorage" of the available calcium and phosphate ions in the end of the growing bone, may play a part in particular cases. Moreover, Schneider and Steenbock have offered evidence of a specific effect of the vitamin upon phosphorus metabolism such as to favor the deposition of calcium phosphate as bone mineral in the cartilage of the developing bone ends, at the expense of the soft tissues, thereby retarding the general growth of the body.

Of interest in this connection is the report of Zetterström that phosphorylated vitamin D activates alkaline phosphatases from intestine, bone, and kidney, thus functioning in the absorption, deposition, and retention of phosphate.

While scientific explanation is probably still incomplete, the problem of prevention of rickets is now sufficiently solved that few new cases of severe rickets are seen, and the mild rickets nowadays occurring may in large part be regarded as incidental to the fluctuating fortunes with which the soft tissues and the developing bones compete for the phosphate which the blood stream brings.

The prevention of rickets by the exposure of the body to sunshine or ultraviolet irradiation undoubtedly comes about through the production of "animal vitamin D" (vitamin D_3) in the skin. Notice, too, the efficiency of the "mechanism" which at the same time increases the circulation of blood through the skin, bringing precursor and taking active vitamin into the body.

The Promotion of Growth and Development

When severe rickets was more common, its outward signs of bowlegs and knock-knees often seemed more noticeable in the more rapidly growing children. This would be natural in so far as general growth means increase of soft tissue; and the more active the soft-tissue growth the less the supply of phosphorus for the deposition of bone salt which is essential to the normal hardening of the growing bones. If the bones are prevented from adequate hardening and

at the same time are made to bear an increasing body weight, the bending of the bones and enlargement of the joints will be worse, the greater the weight put upon them. Under these conditions, then, "the greater the growth, the worse the rickets." But, with the discovery and general use of vitamin D, the situation has largely changed.

What chiefly needs emphasis at present is the importance of liberal nutritional intakes of calcium, of phosphorus, and of vitamin D. When the intakes of calcium and phosphorus are abundant to the needs of the growth and development of both soft tissues and bone, liberality of vitamin D tends to promote growth; more especially linear growth, i.e., increase in the length of the skeleton, perhaps especially of the legs. See especially the work of Stearns, Jeans, and Vandecar (1936) on this effect of vitamin D on linear growth in infancy.

The superior height and erectness of carriage which in some countries has hitherto been considered "a characteristic of the upper class" is due to long straight legs and is now being largely conferred upon the children of all economic conditions through the discovery of vitamin D and the widespread use of fish liver oils. (This is quite as important a service of chemistry to democracy as was Dr. Slosson's favorite illustration, that through the cheap synthesis of the dyestuff "every working-girl can now wear Royal Purple.")

Like some other of the constructive developments of the newer knowledge of nutrition, the favorable influence of liberal intakes of vitamin D upon growth, when intakes of calcium and phosphorus are also liberal, was first shown by experimentation with laboratory animals of short natural life cycle and then confirmed and extended by clinical experience.

In addition to its rôle in the prevention and cure of rickets, vitamin D was found (Sherman and Stiebeling, 1929, 1930) to promote growth and to facilitate the normal calcification in developing bone even when the mineral metabolism is well above the rickets level.

Together with other factors, vitamin D is of importance in the formation of normal teeth and protection against dental caries.

There is clinical evidence that children who have been protected rom rickets by cod liver oil are subsequently less susceptible to espiratory disease than those who have had rickets, but it remains n open question in how far the stronger respiratory system is due o the vitamin D and in how far to the vitamin A which the cod ver oil furnished.

The well controlled clinical investigations of Jeans and his co-orkers seem to bring ever-stronger evidence of the promotion of rowth in children by liberal allowances of vitamin D *when added o diets of liberal calcium and phosphorus content.*

Highly significant is the repeated emphasis laid by Jeans and tearns upon the fact that while vitamin D is in this respect perhaps ven more helpful to physical development than heretofore realized, ne's confidence in the vitamin must never be allowed to make one ess carefully attentive to the importance of liberal calcium intake. ncreased calcification means increased retention of calcium in the ody, and the body must receive large amounts in order to retain arge amounts.

The vitamin must never be expected to decrease the calcium re-uirement. Rather, it helps the body to make optimal use of a gen-rous calcium intake. The intake of calcium must always be generous f optimal calcification and growth of bone are to be secured.

Storage of Vitamin D in the Body and Its Transfer from Mother to Young

Both carefully controlled laboratory experiments with rats and clinical experience with mothers and infants have shown that the antirachitic vitamin can be stored in the body, and to a very important extent. The nutritional condition of the mother influences the storage of vitamin D in the body of the baby before its birth, and her bodily store of this vitamin (or lack of it) affects the antirachitic potency of her milk.

Vitamin D taken by a pregnant or nursing mother is, therefore, a nutritional asset both to herself and to the child.

Hess and coworkers encountered cases in which young rats which

they had intended using as test animals proved "refractory to rickets," because of the highly antirachitic character of the mother's diet and the fact that the litters were small. This, of course, is a converse demonstration of the clinical experience that severe rickets is more frequent among the children of the poor whose mothers are living on food of inferior mineral and vitamin content, often in dark tenements, and also have borne and suckled many children.

Measurement and Expression of Vitamin D Values

Vitamin D values are generally measured either by means of the "line test" or by determination of calcium or total ash in a representative bone.

McCollum and his coworkers have found in their autopsies of rats which had been on deficient diets, that a rickets-producing diet of a sufficiently drastic character may result in a condition in which the cartilage and adjacent portions of the metaphyses of the long bones are entirely free from visible deposits of calcium phosphate. If, then, such rats are given an effective antirachitic treatment of any kind, there results within a few days a line of freshly laid down deposits of calcium phosphate which by proper staining can be made clear to histological examination. This was (and is) called the *line test*. Bills, Honeywell, et al. (1928, 1931) elaborated the technique and gave a very full description of it as used by them. It was also adopted by Steenbock and his coworkers for the routine testing connected with their control of the manufacture of irradiated foods and drugs under the Steenbock patents.

The line test has been adopted as the basis for the *United States Pharmacopeia* unit for vitamin D potency. Present procedure provides for the comparison of the degree of healing caused by the test material with that caused by a known quantity of a standard reference material in a similar group of animals under identical conditions. In this way the effects of variations in the susceptibility to rickets of different groups of rats, due to environmental and other factors, are minimized.

The International standard reference material has been a crystal

line preparation of vitamin D_2; and the International unit has been defined as the antirachitic activity of 0.025 microgram of this standard material. The Expert Committee on Biological Standardization * of the World Health Organization offers instead a crystalline preparation of vitamin D_3, the unit of activity in this case also being defined as that of 0.025 microgram of the reference material. The *United States Pharmacopeia* has adopted this new International standard.

It is well to realize that, in order to produce rickets in rats for the line-test method of assay, greatly unbalanced mineral intakes are employed, the usual rachitogenic diets containing four to five times as much calcium as phosphorus, which is a very much wider ratio than is likely to be encountered in human experience.

Stiebeling has employed a different procedure in which the basal diet fed the experimental animals (rats) is adequate in its mineral content as well as in all organic factors other than vitamin D, and in which the vitamin D value of the material under investigation is judged by the relative amount necessary to allow a standard degree of calcification under fixed experimental conditions.

Young rats receiving the basal diet only (and no irradiation) serve as "negative controls"; those receiving the basal diet plus abundant vitamin D (or irradiation) serve as "positive controls"; others receive the basal diet plus graded allowances of the food or other material which is being tested for vitamin D value.

At the end of a four or five week experimental period the degree of calcification is found by determining the percentage of ash or of calcium in the freshly removed femur. Within certain limits, this is found to be dependent upon the relative amount of vitamin D supplied. The advantage in calcification shown by the test animals over the negative controls must be due to the material being tested, provided the experiments are properly conducted and in sufficient numbers to avoid vitiation of results through individual variation. It is suggested that the "end point" for comparison be one half as much

* World Health Organization: Expert Committee on Biological Standardization. Report of the Sub-committee on Fat-soluble Vitamins. 1949 *Chron. W.H.O.*, 3, 147–148.

improvement in calcification over negative controls as is produce by supplying an abundance of vitamin D (positive controls). Th amount of the food needed to "reach this end point" (maintain thi standard rate of calcification) may be taken as inversely proportiona to its richness in vitamin D.

The Human Requirement of Vitamin D

This section is largely based upon the treatment of the same sub ject by Jeans and Stearns. They consider that for many persons som vitamin D in addition to that ordinarily obtained by exposure t sunshine is necessary for optimal use of calcium and phosphorus i the body. This may be obtained by special irradiation of the skin by use of such foods as contain vitamin D, by the taking of the vita min in some more concentrated form, or in a combination of thes ways.

Jeans and Stearns point out that we should recognize three phase of the vitamin D requirement: (1) that for normal growth and min eralization * of the skeleton, including the teeth, of infants and chil dren; (2) that for maintenance of these skeletal structures in soun condition throughout adult life; and (3) that to supply the specia needs of pregnancy and lactation.

There is as yet no consensus of scientific opinion as to what con stitutes a normal or optimal rate of mineralization of the developing skeleton. Jeans and Stearns state that this is most rapid in early life and that among the estimates of desirable calcium retention "the higher figures are thought to be a safer guide."

During infancy the vitamin D requirement depends to some exten upon the rate of growth. The more rapidly the infant grows the more important it is to safeguard him against rickets: also the libera allowance of vitamin D, given as such a safeguard, itself tends to increase the rate of growth (Jeans and Stearns, 1939, p. 486). Infants

* Mineralization is a useful word to remind us that the calcification of the bone tissue involves a deposition of bone *mineral*. It is worth while to preserve the distinction (sometimes neglected in nutritional writings) between a minera and a mineral element. The iron of hemoglobin, for instance, is a mineral ele ment but not a minera'.

whose daily food includes a quart of milk containing 135 units of vitamin D seem to be fully protected from rickets; but additional vitamin D up to a total of 300 to 400 units seems to support a rate of growth and development "nearer the top of the normal range." See chart quoted by Jeans and Stearns (1939, p. 488) from Slyker, Hamil, Poole, Cooley, and Macy: *Proc. Soc. Exptl. Biol. Med.* 37, 499.

It also appears, from the observations of Jeans and Stearns and of Macy and coworkers, that increasing allowances up to an undetermined level between 135 and 300 units of vitamin D per day tends toward a more uniform ability among infants to assimilate calcium from the food. This appears to be true even of infants fed human milk (Jeans and Stearns, 1939, p. 493).

During childhood there is need of continued emphasis upon liberal intakes of calcium and of vitamin D if optimal development of bones and teeth is to be maintained. According to Jeans and Stearns (1939) the needs of children in this respect do not seem to have been as fully realized as the needs of infants, probably because it is less easy to make the child's need obvious. Beyond emphasizing the view that there should be no let-down of calcium or vitamin D intake as infancy develops into childhood, little can yet be said as to the child's quantitative need.

Jeans and Stearns have studied fifty children aged 1 to 12 years with varying intakes of milk and with and without added vitamin D in the form of cod liver oil to the extent of 300 to 400 added units of vitamin D per day. They found that children varied in their need. If a child utilized calcium efficiently without the added vitamin D, the addition of this to the diet had little effect; while if the original utilization was poor, the ingestion of additional vitamin D increased the retention of calcium.

Whether adults need vitamin D and if so to what extent are questions upon which there is little evidence beyond the fact, so carefully and repeatedly emphasized by Jeans and Stearns, that vitamin D does not lower the minimum requirement for ingested calcium.

In pregnancy and lactation it is undoubtedly wise to be liberal with the vitamin D allowance.

The National Research Council's Recommended Allowances provide 400 units per day for each child or pregnant or nursing woman.

Sources of Vitamin D

The antirachitic values of fish liver oils, of irradiated ergosterol, and of ultraviolet irradiation of the body, are so great that they have absorbed attention to perhaps a greater extent than is desirable. The presence of important amounts of vitamin D in egg yolk, whole milk, and butterfat has, however, been established. That in some cases the evidence of its presence has failed to appear is probably due in large part to the use in many experiments of so drastic a rickets-producing diet that symptoms of rickets developed in the experimental animals notwithstanding the presence of amounts of vitamin D (in the food fed for test) which would have been measurable under more nearly normal conditions. Mellanby, working with puppies and using basal diets not so drastically deficient as are the rickets-producing diets employed by most of those who work with rats, had no difficulty in showing the vitamin D value of green vegetables and of butterfat. Shipley, Kinney, and McCollum (1924) showed the presence of vitamin D in ether extracts of green leaves. Also in the work of Sherman and Steibeling which demonstrated a considerable vitamin D value in milk, the diets were so arranged and controlled that the effect of the milk in promoting deposition of calcium phosphate in the growing bone must have been due to the vitamin rather than the calcium or phosphorus factor of the food, since the amounts and ratios of calcium and phosphorus in the intake were strictly controlled.

As was seen to be true of vitamin A, so also with vitamin D there seems to be survival value for the species in the capacities that have been developed by the maternal organism to collect the vitamin and pass it on to its young through the fat of the egg yolk or of the milk as the case may be. The concentration is higher in the egg fat, but the total amount of vitamin D obtained through the milk fat is considerable because of the consumption of the latter not only in the form of milk itself but also of cheese, butter, cream, and ice

cream. Vitamin D is sufficiently stable, except under irradiation, so that no serious loss need be feared in the making or storing of such foods as these, or in ordinary cooking operations. Direct experimentation has shown its stability at autoclave temperatures.

Vitamin D milk is the term commonly used for market milk of enhanced vitamin D value.

Investigators differ as to the practicability of materially increasing the vitamin D value of milk by the irradiation of the cow.

Three methods of enrichment of milk in vitamin D have been in practical use: (1) the addition of a concentrate of natural vitamin D; (2) feeding irradiated material to the milch cow; (3) irradiation of the milk itself.

The choice between these three methods depends partly on economic factors, and partly upon considerations having to do with the differences in kinds and relative proportions of the vitamin D or vitamins D introduced by the respective methods.

Whatever may be the ultimate consensus of opinion as to the relative merits of these different forms of enrichment, it is already established that vitamin D milk is a highly effective means of administering vitamin D.

With milk of such greatly enhanced vitamin D value now generally available, and with the wide general distribution of cod liver oil, of the more potent liver oil of the halibut, and of the still more potent *percomorph liver oil*, it is proper enough to place chief dependence upon these highly potent and now well standardized sources. But the natural vitamin D values of foods are scientifically significant to our understanding of the processes of nature and how children got along even as well as they did before vitamin D was discovered.

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Chapter 25

OTHER FAT–SOLUBLE VITAMINS

Vitamin E (Tocopherols)

Almost simultaneously with the full establishment of vitamin D as a fat-soluble essential distinct from vitamin A, different investigators, prominent among whom were Evans and his coworkers at the University of California and Mattill, working first at the University of Rochester and later at Iowa University, found evidence that for normal reproduction a further fat-soluble factor was nutritionally essential. This came to be called vitamin E, then *tocopherol* and more recently *alpha-, beta-, gamma-,* and *delta-tocopherols.*

The four known natural tocopherols are so closely related that custom continues to use the collective-singular term, vitamin E.

Synthetic alpha-tocopherol acetate has been adopted as the International standard for vitamin E; and the International Unit has been defined as the specific activity of 1 milligram of the standard preparation. Vitamin E is rather widely distributed in nature where it is largely associated with anti-oxidants (Bradway and Mattill, 1934; Olcott and Mattill, 1931, 1934).

Chemically, this association with anti-oxidants in natural occurrence correlates with the fact that vitamin E, while relatively stable in several respects, is rather easily destroyed by oxidation.

The oxidative destruction of vitamin E is catalyzed by iron salts. Hence a food mixture for experimental purposes can be made deficient in vitamin E by moistening with a solution of iron salt and then heating to dryness.

When rats are fed diets otherwise adequate to their needs but lacking vitamin E, the males become permanently infertile through an irreversible degeneration of the germinal epithelium; females fa

reproduction, the embryos being resorbed, but the female reproductive system does not suffer permanent injury.

The effects of a lack of vitamin E are not, however, limited to the reproductive organs. The symptoms most prominent vary from species to species, and with the age of the individual, but in one or another species may include failure of growth, muscular dystrophy, injury to the central nervous system, interference with normal heart action, and possible endocrine disturbances.

Whether the counterpart of any of these conditions occurs in human beings is still controversial.

Evans (1939) pointed out the occurrence of degeneration of the cross-striated musculature in vitamin E deficiency. It has also been suggested that this may be the cause of the failure of development of the fetus. Compare Mason (1944).

Puppies, rabbits, guineapigs, and rats have developed muscular dystrophy when kept for long periods on vitamin-E deficient diets, and in some cases this has been cured by giving synthetic alpha-tocopherol.

Vitamin E has also been found to have in some sense a sparing effect upon vitamin A. The converse may also be true, as described, for example, in the papers of Hickman, et al. (1942, 1944), and Harris, et al. (1944) and others among suggested readings listed below.

Vitamin E occurs abundantly in wheat germ oil, but may not be its only influential constituent as has been assumed by some experimenters. Other vegetable oils also are rich sources of vitamin E.

Because of its wide distribution in foods and the improbability of its being a limiting factor in human nutrition, it seems probably unnecessary and possibly misleading to lay emphasis upon vitamin E in practical considerations of food values. Reports of cases in which vitamin E has seemed to be helpful in the treatment of certain muscular and nervous diseases are included among the references at the end of this chapter; but it should be noted carefully that the Council on Pharmacy and Chemistry of the American Medical Association has warned against regarding the value of vitamin E for man as

established, and has emphasized the need of more fully controlled investigation.

Vitamins K_1 and K_2

As early as 1929 it had been observed by the Danish investigator Dam that chickens raised on certain artificial diets were subject to subcutaneous and intramuscular hemorrhages; and further study of such a hemorrhagic chick showed an abnormally long time required for the clotting of the blood. Others confirmed these observations and Dam (1935) extended his work to the finding of experimental evidence that the hemorrhagic tendency and abnormally prolonged clotting time were attributable to shortage of a fat-soluble "vitamin K" (from the Scandinavian and German term, "Koagulations-Vitamin").

The work of several investigators showed that such a factor is widely distributed in green leaves.

One form of antihemorrhagic vitamin, obtained from alfalfa leaf meal and designated as vitamin K_1 or as phyllochinon (phylloquinone), has been identified chemically as 2-methyl-3-phytyl-1,4-naphthoquinone.

A second form of antihemorrhagic vitamin (vitamin K_2) was isolated by Doisy and coworkers from putrefied sardine meal (Binkley, McKee, Thayer, and Doisy, 1940).

In addition to these forms of vitamin K known to occur naturally, many synthetic products show antihemorrhagic properties in greater or lesser degree. References to numerous investigations of this subject are to be found at the end of the chapter.

Bile salts appear to be highly important if not absolutely necessary to the absorption of (natural) vitamin K. For this reason, patients with diseases of the biliary tract in which bile flow to the intestine is impaired are apt to develop a condition of low blood clotting ability which is really a K-avitaminosis, notwithstanding the fact that their diet may have contained normally adequate amounts of antihemorrhagic factor. Numerous clinical tests show that the hemorrhagic tendency in such conditions may be controlled either

by the oral administration of bile salts with vitamin K concentrates or by the injection of antihemorrhagic vitamin.

The low clotting-power of the blood in vitamin K deficiency has been traced to an abnormally low content of *prothrombin* (a normal protein constituent of the blood which, with calcium ion and the cephalin-containing thromboplastic factor, gives rise to blood clot). Hypoprothrombinemia has been found clinically in various conditions of impaired digestive function and treated effectively in most instances as a K-avitaminosis. Since diminished clotting power due to low prothrombin content appears to be usual in the blood of newborn infants, clinicians generally recommend administration of vitamin K either to the mother before delivery or to the infant at birth.

For fuller accounts, see the excellent reviews of Dam (1940) and of Brinkhous (1940) and later papers listed below.

Stamler, Tidrick, and Warner (1943) have reported * upon vitamin K and prothrombin levels with reference to the influence of age and of rapidity of growth.

The National Research Council's circular on *Recommended Dietary Allowances,* Revised 1948, comments upon vitamin K in part as follows:

"The requirement for vitamin K usually is satisfied by any good diet except for the infant in utero and for the first few days after birth. Supplemental vitamin K is recommended during the last month of pregnancy. When it has not been given in this manner, it is recommended for the mother preceding delivery or for the baby immediately after birth. . . . Pregnant women usually are found to have normal amounts of prothrombin in the blood, but many newborn infants have abnormally small amounts. Although little relation seems to exist between the amount of prothrombin in the blood of the mother and that of the infant at birth, the amount of vitamin K received by the mother is reflected quickly in the amount of prothrombin in the blood of her infant in utero. Seldom does the mother receive enough dietary vitamin K to prevent an important decrease in the prothrombin level of the blood of her infant during the first few days after birth. . . . The available evidence warrants increased attention to the vitamin K intake of the mother during the latter part of

* *Journal of Nutrition,* Vol. 26, pages 95–103 (1943).

pregnancy . . . (and) it is suggested that vitamin K be given to preg nant women during the last month of pregnancy."

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VITAMIN E (TOCOPHEROLS)

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apter 26

THE NUTRITIONAL CHEMISTRY OF REPRODUCTION AND LACTATION

Nutritional Needs of Pregnancy

We do not know that the food of the expectant mother need furish any different thing from the nutrients required for growth and aintenance, but it is clear that the function of gestation increases e nutritional need.

To cite only two of the researches which amply support this fact, may be emphasized that both at the Harvard Medical School and Toronto, investigations have shown that both mother and child rofit greatly by superior feeding during pregnancy (Burke, Beal, irkwood, and Stuart, 1943, 1943 *b;* Ebbs, et al., 1941, 1942). And lendenhall has written that, "To count on the fact that the mother a factor of safety in the nutrition of the young, and to nourish the hild at the expense of the pregnant or nursing mother is an unnecesary sacrifice of the woman and may at any time prove disastrous to ne child." And also, "There is every reason to believe that ability o produce breast milk of a superior quality is to some extent dependent on the storage of material from the mother's food during ne prenatal period, as well as on the supply of nutrients from the ood she receives during lactation."

The increase in the energy metabolism becomes measurable after bout four months of pregnancy and then rises steadily until at term t is about 25 per cent greater than for the same woman during reproductive rest. (See Chapters 10 and 28.)

In submitting its recommendations of 1948, the National Research Council stated that "as to the most desirable levels of calorie intake during pregnancy and lactation, the allowances recommended . . . seek a middle ground within the somewhat broad range of present professional opinion."

Hunscher, et al. (1935) found that with generous amounts of proteins in the diet, the maternal organism tended to store nitrogen greatly in excess of the amounts retained in the fetus and its adnexa; and that this maternal reserve acted to offset the strongly negative nitrogen balances of the subsequent (in the case which they studied, unusually heavy) lactation period.

On the other hand, Coons has found in pregnant women who chose diets considerably lower than usual in protein content (about 11 grams of nitrogen a day) that the retentions of nitrogen were not in all cases as high as the amount presumably transferred to the fetus; and there were indications that low storage of nitrogen during pregnancy might adversely affect lactation.

The amounts of material actually transferred into the body of the fetus are of distinct interest here. As an average of rather widely varying reports, it appears that about 60 grams of nitrogen, 20 grams of calcium, and 15 grams of phosphorus are contained in the body of the normal infant at birth.

Obviously, if the mother's positive balances during pregnancy should fall short of the amounts with which the baby is born, the maternal organism must have been depleted. This is, perhaps, more often true of calcium than of other elements thus far studied. Thus Coons and Blunt (1930) found that a group of Chicago women, each choosing her own diet, stored during pregnancy less calcium than the fetus was estimated to contain. Moreover, it does not follow that the materials contained in the body of the baby at birth represent the whole of the increased need of pregnancy over simple maintenance. Macy and Hunscher (1934) have computed that under favorable nutritional conditions, the positive balances during pregnancy amount to about 500 grams of nitrogen, about 65 grams of phosphorus, and about 29 grams of calcium (or 700–800 per cent more of nitrogen, 300–400 per cent more of phosphorus, and about 25–50 per cent more of calcium than is contained in the body of the baby at birth). To an extent not yet clearly defined, these surpluses appear to represent the laying up by the mother during pregnancy of nutritional reserves against the heavy demands of lactation. Macy and coworkers (Hummel, et al., 1936) show by prolonged balance

tudies the practicability of providing through the food for such lib-ral storage of protein, calcium, magnesium, and phosphorus.

The increased need for iron during pregnancy has been explained n Chapter 15 in general terms. An attempt to treat it in a more quantitative way would probably be premature, as the estimates of he amounts contained in the normal fetus and retained during a ormal pregnancy are as yet too variable to afford a sound basis for nterpretation until many more cases have been studied.

In regions in which the environment provides no liberal margin f iodide intake (in general, the goitrous regions), iodine deficiency nay be induced by the extra demands of pregnancy and lactation. Medical advice should be sought in safeguarding against this danger.

Vitamin requirements are undoubtedly increased in pregnancy, nd we have no ground for assuming that the increase is the same r closely similar in the case of each vitamin. Abundance of vitamin A has been shown to be very beneficial.

Division of the Penalty of Malnutrition between the Mother and the Offspring

To a large extent, the organism of the mother serves as a "factor f safety" in the nutrition of the young both before birth and during actation. Occasionally, however, this important principle,—that Na-ure is "more careful" of the perpetuation of the race than of the onservation of the adult—may be overemphasized. Thus it is an xaggeration to say that the unborn offspring "is a perfect parasite pon the mother." It is in a sense a "parasite" but not a "perfect" one n the sense of taking just as much from the mother whether she has to spare or not.

The fetus may draw heavily upon the mother, taking material be-ond that which the mother can well spare but when the nutritional ntake is seriously deficient to the total demand of pregnancy it must e expected that both the mother and the young will be unfavorably ffected.

This is illustrated in the classic experiment upon young milch

cattle at the University of Wisconsin. Four groups of young females were placed on four rations derived respectively from the wheat plant, the oat plant, the corn (maize) plant, and a mixture of the three. By mixing proper proportions of the leaves and stalk of the plant with its seed (and when necessary supplementing with some of a protein-concentrate of the same seed) all four rations were given the same energy and protein content. Growth was completed on all four of these rations; but the reproduction and lactation records were very different. Here, because pregnancy and lactation imposed such heavy nutritional demands, a diet which was satisfactory even for growth fell short of the needs for satisfactory bearing and suckling of young. Thus, support of reproduction and lactation becomes a delicate test of the quality of a diet. The use of the reproduction and lactation records, as an important criterion in a connected series of criteria of improvement in nutritional status, is well illustrated in the work of Campbell (1928, 1931).

Nutritional Support of Lactation in the Human Mother

Direct experimentation upon milk production has usually shown that not more than two thirds of the extra calories fed are recovered in the milk, or conversely, that the extra food requirement (in calories) is at least 50 per cent greater than the number of extra calories in the milk produced. In the work of Shukers, Macy, et al. (1931) it was found that: "Lactation increases the food demands (of the human mother) approximately 60 per cent over and above those of pregnancy." The actual voluntary food intakes found in two unusually successfully lactating women were 4600 and 4800 Calories per day, respectively.

In cases in which the physician believes that liberal feeding of the mother may help in establishing the activity or promoting the productivity of her mammary glands, it is doubtless wise (at least until knowledge in this field becomes more precise) to make the dietary liberal in respect to all nutritional factors; for the consensus of competent opinion is clearly in favor of the nursing of the baby by the

other, in all reasonably normal cases. And physicians are increasgly successful in stimulating human milk secretion.

Yet it may also be said that something in human evolution or in the nditions of present-day civilization seems to have worked at some st of the natural function of human lactation, so that even with e best of nutrition the mother may not secrete as much milk as e baby needs. When the physician is thoroughly convinced that is is true of the individual case, the fact may be accepted without y undue feeling of defeat.

Liberal feeding for the support of lactation should begin early; for can safely be inferred from the results of animal experimentation at the ability to sustain a good rate of milk production depends gely upon the nutritional condition induced and maintained by od feeding from the beginning of the lactation period.

For the *effects on milk production of deficiencies in the food pply*, the reader is referred to Meigs' excellent discussion of this pic in the treatise entitled, *Fundamentals of Dairy Science*, by the sociates of Rogers, which should be read in full by those who are terested in this aspect of lactation.

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Chapter 27

SOME CHEMICAL ASPECTS OF GROWTH AND DEVELOPMENT

In the first decade of our century, Waters used the words which have been so frequently quoted that they may be taken as expressing the point of view which prevailed for a generation. "The upper limit of the size of an animal is determined by heredity. The stature to which an animal may actually attain, within this definitely fixed limit, is directly related to the way in which it is nourished during its growing period.".

Subsequent scientific evidence has somewhat modified the view that heredity predetermines the limit of an individual's or a family's development in quite such a "definitely fixed" manner. Like many other useful generalizations in science, Waters' formulation above quoted comes to require modification in order to be consistent with new scientific knowledge in its field.

The increased growth which Mendel and Hubbell (1935) found to progress through many generations of their animal colony could not have been predicted from the heredity of these animals; and that it was a phase of a real improvement in these families is indicated by the fact that these same animals showed also an enhancement of adult vitality or stamina as reflected in the success of their reproduction and rearing of young.

Similarly the improvement of an already adequate diet by Sherman and Campbell (1924, 1930, 1935) likewise proved favorable both to rate of growth, and to adult vitality, and in this case it was also shown to increase the length of adult life, and to extend by a larger percentage the period between the attainment of maturity and the onset of senility.

Todd (1935) wrote: "The adult physical pattern is the outcome of growth along lines determined by heredity but enhanced, dwarfed,

arped or mutilated in its expression by the influence of environ-
ent in the adventures of life."

Speaking with extreme reserve as President of the Royal Society,
opkins pointed out that "nurture can assist nature" to a larger ex-
nt than has been supposed by the scientific thought of the past
vo generations.

Effects of the Levels of Intake of Different Nutritional Factors upon Growth and Development

In whatever terms one chooses to state the change in our concepts
ferred to in the preceding section, the outstanding significance of
e change is an awakening to the fact that the life history depends
ore upon the choice and use of food than hitherto believed.

Nutrition need not be merely a matter of supplying the predeter-
ined needs of an inherited mechanism. It can be more constructive
an that. Thus it is something which in the light of our newest
owledge is seen to bear more responsibility than previous gen-
ations supposed.

There is need to discriminate among the different factors in nutri-
on: of some factors "enough is as good as a feast," while of other
ctors more generous surpluses contribute to a better outcome. Also,
course, shortages of different factors produce different results.

As explained in earlier chapters, it was shown by Osborne and
endel that, with a diet adequate in all other respects, any one of a
mber of purified proteins such as casein, lactalbumin, or edestin
ay, if used in sufficient amounts, serve as the sole protein both for
aintenance and for growth, while gliadin as sole protein food suf-
ed for maintenance but not for growth, and zein as sole protein
d not suffice even for maintenance. Gliadin contains adequate
yptophane but only about 1 per cent of lysine; addition of more
sine to the gliadin ration made it adequate for growth. Hence the
ilure to grow when gliadin is the sole protein in an otherwise ade-
ate diet is a specific stunting due to shortage of lysine.

When growth is retarded by inadequate intake of this particular
mino acid, the emaciated appearance characteristic of animals at-

tempting to grow on an insufficient energy intake is not to be expected. Osborne and Mendel have recorded numerous cases of suspension of growth of young rats, especially when kept on rations containing gliadin as a sole protein food. Here the inadequacy of the lysine intake results in retardation or even complete suspension of growth, but the animal may remain quite healthy and symmetrical. Moreover rats may be subjected to this type of stunting for a remarkably long time and still retain their capacity to grow when given an adequate diet.

In contrast to the striking extent to which lysine deficiency may result in simple suspension of growth without apparent injury, it has repeatedly been shown that a tryptophane deficiency is much more likely to be disastrous; while the cystine stunting described by Woods (1925), like the lysine stunting of Osborne and Mendel, resulted in retardation or suspension of growth with no evidence of a pathological condition.

Hence it is plain that the results of protein stunting may be different according to the particular amino acid shortage which is involved. In general, however, the evidence indicates that stunting from shortage of protein is apt to involve less injury than other forms of stunting.

Because the normal calcification of children's bones often lags behind other phases of their bodily development, much experimentation has been devoted to the problem of the relations of age, growth, and food to the body's increase of its percentage of calcium. Calcium-balance experiments with children, from which their increases of body calcium percentage can be calculated, have been considered in Chapter 14. As another means of study of the calcium retention, extensive series of analyses have been made of rats of known hereditary and nutritional history, which have been differently fed under controlled conditions, or which have made different rates of growth on the same diet. In such experiments by Briwa, by Conner and Kao, and by Lanford and Campbell in the writer's laboratory, it has been found that neither more rapid growth as an individual characteristic nor the increase of the growth rate by enrichment of the dietary in its protein content augments the rate of increase of per-

entage of calcium in the body; but that this is augmented by enchment in the calcium content of the diet (in experiments with ats, up to about 0.6–0.8 per cent of calcium in the dry weight of the od).

The findings outlined by the preceding paragraphs illustrate the istinction emphasized by Todd between what he considered mere rowth, on the one hand, and, on the other hand, development of the aturation pattern of the skeletal tissue with increase in the *perentage* of bone mineral which it contains. (We cannot here pursue he further point that there need not always be exact parallelism beween calcium percentage and the details of the Roentgenograms.)

By analyses of relatively large numbers at each of several ages, it as been found that rats of families on food of optimal calcium conent (0.64 to 0.80 per cent of the dry food mixture) attain at an verage of one month of age a percentage of body calcium which is ttained only at a much later age by their cousins in families whose od is of only about minimal-adequate calcium content, i.e., 0.20 er cent of calcium in the dry food mixture (Lanford, Campbell, nd Sherman, 1941).

Moreover, the families living upon the more liberal calcium level owed higher vitality and lower death rates at all stages of the life ycle.

This brings us to a question which has been much misunderstood, amely, whether rapid growth is in itself an influence tending to hortening of life. To study this question with human beings as the ubjects, is impracticable for two reasons: the investigator could not ount upon his own life cycle being long enough to enable him to arry his research project through; and normal human beings canot be so controlled throughout their lifetimes that only one enironmental factor can vary. Rats, however, are strikingly like "us umans" in most aspects of their nutrition and yet they complete heir normal life cycles in only about one-thirtieth of a human lifeme. Rats have, therefore, been chosen as the subjects for the exerimental study of the relation, if any, between rate of growth and ngth of life. Hundreds of well matched twins have been compared rom infancy until the ends of their natural lives (*a*) with their diets

as well as all other factors alike, and (*b*) with all genetic and environmental factors alike *except* for some one quantitatively controlled food factor.

When *all* environmental factors were uniform so that differences in growth were due *only* to the individual growth impulse, those which grew faster and those which grew more slowly were found to have equally good chances of long life.

On the other hand, when a controlled food factor was different and this difference resulted in either a slower or a more rapid growth, then the length of life may or may not be influenced, and either in the same direction as the difference of growth rate or in the opposite direction. Instances of both kinds are summarized in Table 64 (Chapter 34).

To an even greater extent than body iron is localized in the blood, the calcium of the body is localized in the bones and teeth. The skeletal system contains over 99 per cent of the body calcium. A low intake of calcium during growth retards the development and calcification of the bones and teeth. Such development may also be interfered with by inadequacy of the phosphorus supply, especially at early ages when the muscles are growing rapidly and competing with the bones for the phosphorus which the blood brings. Thus the low-phosphorus type of rickets appears to be more common than the low-calcium type; but considering the period of growth as a whole, there is greater danger of shortage of calcium than of phosphorus. Several investigators, in studying the effect of diet upon growth of bone, have found that the bones formed in a young animal kept on a diet poor in phosphorus and calcium are apt to be soft, spongy, and weak, and that this may be prevented by the simple addition of calcium phosphate to the food. Confidence in the value of the antirachitic vitamin must not lead to any lack of care in providing a liberal supply of calcium and phosphorus during growth. For, whatever aid we may secure, through vitamins or otherwise, in maintaining a favorable condition for growth of bone, calcium and phosphorus are the chief elements which must always be supplied and retained in liberal amounts as actual constituents of the skeletal framework of the body.

During the decades in which artificially refined foods were increasingly used and the importance of the protective foods had not yet become known, calcium intakes were doubtless very often suboptimal. Readjustment under the guidance of the newer knowledge of nutrition is now in progress; but even recent calcium-balance studies with American children indicate that an undue proportion of our child population is apt to lag in this phase of normal development, and that continued emphasis upon the importance of greater prominence of calcium-rich food is still needed in many if not most American communities.

McCollum has conducted a controlled experiment in an orphanage and found that the addition of a quart of milk a day to the diet of each of 42 children resulted in improvements in their rate of growth and in their general well-being, quite as indicated by experimentation upon laboratory animals. Here the original diet may have been visibly in need of improvement even without experimental demonstration; but in the investigation conducted by Corry Mann the starting point was a dietary which was already fairly good. The quantitative aspect already mentioned deserves further note.

Mann (1926) worked during a period of four years in an English institution for boys. The boys were between 7 and 11 years old. They were divided into seven groups, carefully matched as to age and size. The first group received the regular diet "originally chosen with every regard for the welfare of the children to be reared upon it"; and the second group was given, in addition, one pint of milk per capita daily; the third, sugar equivalent in calories to the pint of milk; the fourth, butter from grass-fed cows in such amount as to furnish the same number of calories as the milk; the fifth, an equivalent amount of vegetable margarine; the sixth, sixty-five Calories of edible casein; and the seventh, three fourths of an ounce of fresh watercress per day. The milk group made the largest gains, viz. 6.98 pounds and 2.63 inches a year each; the butter group came second with 6.3 pounds and 2.2 inches; and the watercress group came third, indicating that extra vitamin (especially vitamin A) was more important in these cases than extra calories or extra protein even when the latter was of such excellent a kind as casein. Sir Walter M.

Fletcher, eminent English physician and member of the British Medical Research Council, emphasized strongly the significance of this work as showing that even on a dietary "medically adjudged to be sufficient for healthy development, the boys were in fact not attaining to the physical and mental growth of which they had the potentiality, and to which they did attain when given an extra daily ration of milk." And he pleaded that no other improvement, however desirable in itself, should be given priority over better nutrition: "First things should come first."

Orr,* and also Leighton and Clark,† have shown on a large scale that addition of milk to the diet of school children has a markedly larger influence upon growth in both height and weight than does the addition of an equal number of calories in such other form as biscuit; and also that the children getting the additional milk showed a superiority of "condition" which is not readily defined, but is recognizable as standing for something beyond pounds and inches; and also these children showed greater alertness and buoyancy of spirits.

It is important to recognize that even if the better feeding can not be permanent, it yet may confer a permanent benefit. In a conference looking to nutritional improvement of the children of Puerto Rico, the question was raised whether it would be wise to introduce milk into the school lunch when it was not certain that the milk supply would continue. "Yes," rightly said Dr. McKinley, "for every day that the child *does* get the milk, he puts calcium and vitamin A in his bank."

Moreover, the material "banked" is not simply laid by as a passive reserve. In putting the body forward in development it becomes a permanent addition to the bodily "working capital," as in the better bone development observed by MacNair (1939) to result from the addition of cod liver oil even to a diet which appeared to be good. Both vitamins A and D probably contributed to this improvement.

Boyd, Drain, and Nelson among others have shown that an increased use of milk, fruit, and vegetables in the dietaries of children is followed by a great reduction in the occurrence and severity of

* *Lancet* **1928, I,** 202
† *Lancet* **1929, I,** 40.

ental caries; though Bunting has questioned whether this unoubted effect of food is to be interpreted on nutritional or on bacteriological grounds.

Boyd, Drain, and Stearns (1933) have further confirmed the fact hat human dental caries is in general (though perhaps not in every ndividual case) related to the food; and they find a definite and ignificant relation between the level of retention of calcium and hosphorus and the resistance of the teeth to decay. They emphasize he view that for optimal results there is need of higher calcium etention than has usually been accepted as adequate.

Any of the many aspects of growth and development may be influnced by seasonal relationships. Temperatures which increase energy netabolism, either directly or through encouraging more exercise nd higher tonus of the muscles, naturally induce the eating of a arger amount of food.

Benedict and MacLeod found that the heat production of rats was listinctly higher at 25.7° C. than at 28.9° C., the "critical temperaure" for this species being apparently about 28° C. Correspondingly he heat production averaged 10 to 12 per cent lower in summer than n winter. In harmony with this is the observation of Campbell that ood intake expressed in calories per gram of rat per day is lower in ummer than in winter. And in this connection it is also of interest hat Gloy found higher growth rates (among these rats) in winter han in summer. Eating more food to supply their greater calorie eed in winter, they thereby increase their intake of proteins, minral elements, and vitamins.

Similarly children's appetites and energy requirements may be ncreased by exercise and, with the diet well balanced, this will nean, along with the increased intake of calories, a correspondingly ncreased intake of all the growth essentials. Hence exercise and ood feeding act together in promoting the development of the child.

Growth Experiments as a Means of Research

Previous chapters have afforded illustrations of the use of growth xperiments as means of investigation in many different branches or

aspects of the chemistry of food and nutrition. So widely is this means of research now used, that it has become important to consider with some care the quantitative applicability of the usual statistical interpretation of the experimental data thus obtained.

Here the primary question is, Will the variations in data of like feeding experiments show a normal frequency distribution, i.e., when plotted will they resemble a symmetrical bell-shaped curve,

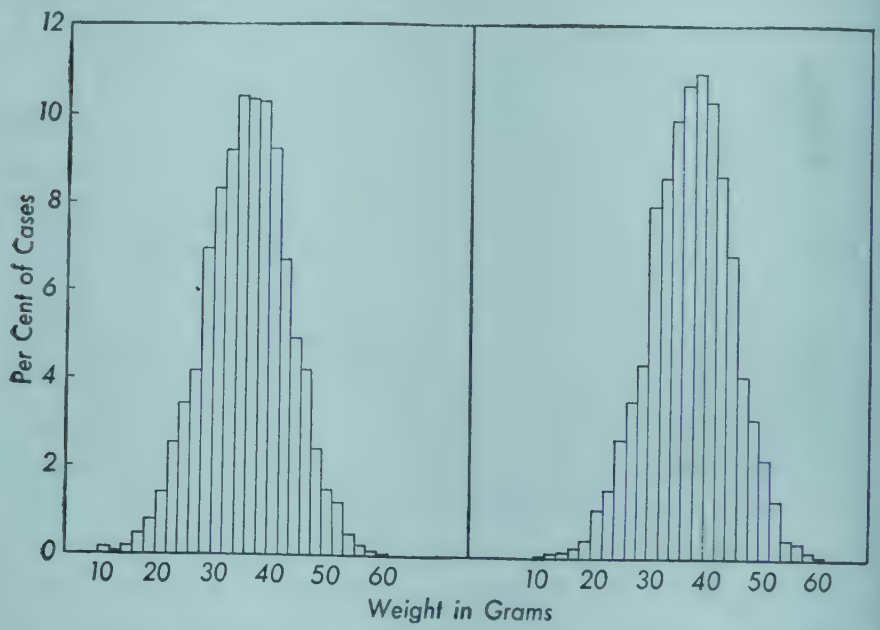

FIG. 24. Frequency diagram for weights of rats, of same hereditary and nutritional history, at 28 days of age. On the left, data of 3092 males, skewness—0.04; on the right, data of 3243 females, skewness—0.04. (Courtesy of the *Proceedings of the National Academy of Sciences.*)

so that we may justifiably feel confident in applying the usual quantitative interpretation to such data?

Since the work of Rietz and Mitchell (1908) this has seemed sufficiently probable to warrant the use of the ordinary statistical interpretation of such data. There are now at hand extended series of measurements of growth which actually demonstrate a very satisfactory symmetry in the frequency distributions, as shown in Figure 24, and justify a high degree of confidence in the use of the classical methods of simple statistical interpretation, such as the computation of Standard Errors and of Probable Errors of the mean

sults of series and of the difference between the means of two ries, and the numerical expression of the probability or "chances" at an observed difference is statistically significant (Sherman and ampbell, 1934).

Rietz and Mitchell (1910) wrote: "It is a generally recognized fact at two men to all appearances under identical conditions . . . . ill often on experimental investigation, yield considerably discordnt results as to utilization of the food . . . changes in body weight nd perhaps more especially, structural composition of the blood, . . rate and character of digestion, etc." . . .

Such physiological behavior, peculiar to each individual organm and neither amenable to prediction or control, is ordinarily colctively designated as "individual idiosyncrasy," whereas Rietz and Mitchell prefer the expression "functional variability" and state that is is what makes "the problems of metabolism statistical." They oint out that many classes of things and processes, of different naure and origin, . . . conform to one and the same law, *Gauss's Law of Error*" (the law of normal frequency distribution). A funamental consideration is therefore whether the kind of thing or of rocess is such that a charting of frequency distribution resembles ne bell-shaped curve. That this is the case in our use of growth ata as a means of research in nutritional problems, such as are dealt ith in comparisons of nutritive values of foods and of diets, is ilustrated in Figure 24. The extreme slightness of the skewness of nese bell-shaped curves adds much to the confidence with which we ay use this general method of investigation when sufficiently large umbers and quantitative controls are employed. The very fact of hysiological or functional variability implies the importance of arge numbers and strict controls, preferably in the hands of workers ith a background of training in the exact sciences. In such hands, nd extended to sufficiently large numbers of subjects sometimes ontinued throughout entire growing periods or even entire lives of uccessive generations, the investigator with a laboratory colony of uitable experimental animals can now enjoy a new order of condence over that attaching to the "mere handfuls of men" to which ietz and Mitchell's warnings apply.

Notwithstanding the somewhat pessimistic cautiousness of much of their paper (chiefly because they are usually thinking of experiments with small numbers) Rietz and Mitchell conclude that they "do not hesitate to predict that by the use of such (statistical) methods, a greater degree of exactness and precision . . . will result, fully repaying the extra labor and care involved in conforming to the requirements of these methods." This optimistic viewpoint is strongly supported by the excellent agreement of the growth data of Figure 24 with the law of Gauss or the principle of normal distribution of data.

Superior Nutrition and Mental Development

As early as 1925, Baldwin found a parallelism of mental and physical development in the superior children that he studied.

In several of the publications listed below as Suggested Readings, one can find discussions of the question of the degree to which mental and physical growth may be expected to run parallel, and more casual references to the influence of nutrition upon these two phases of development. Correlation of the evidence to be found in the readings is here left as an exercise for the individual student.

Importance of adequate iodine to both physical and mental development, is, of course, now long-established (Chapter 16).

On the broader question of general mental and physical development, the *Journal of the American Medical Association* has said editorially: "In the absence of more definite correlation between the psychic and the physical, it is well to strive for ideal growth of the body and thus provide the most favorable sphere for the development of the mind."

But also, as other editorials in the same Journal have pointed out, especially in commenting upon the work of Wetzel (1932–1934), ideal growth is not necessarily the most rapid growth that can be induced. Rather it is the growth-rate induced by the plan of feeding which yields the best results for the life-cycle as a whole. This we are learning to define. Certain first-findings of Cornell and Columbia investigators, respectively, which on superficial view may seem to

liffer in their trend, are not to be interpreted as discordant but ather as illustrating the complexity and importance of this field of utritional research.

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Chapter 28

DIETARY ADEQUACY AND NUTRITIONAL STATUS

Even the title of this chapter presents us a problem. The early editions of this book used the term Dietary Standards—a term which was formerly much used in food planning and nutrition teaching but which now seems to carry an undue connotation of finality or rigidity. But if *standard* is too stiff a word, *adequacy* may be somewhat ambiguous. It calls upon us to be always asking ourselves: Adequate for what? Yet perhaps the habit of carrying this question in mind is just what we most need in undertaking the sort of judgments with which this chapter deals. For example, Heilbron, Jones, and Bacharach, writing from the viewpoint of studies of vitamin A requirements, have pointed out that the amount just necessary to prevent overt signs of deficiency is certainly less than that required to maintain "normal health," and that many students of the problem believe this latter quantity to be again less than the amount required for "buoyant," "abounding," "positive," or "optimal" health.

The *Journal of the American Medical Association* has remarked editorially that "the difference between merely passable health and buoyant health is coming to be more appreciated."

Two facts need emphasis here as elsewhere: (1) that even the distinction between normal and subnormal will fall at different levels, depending upon the delicacy of the diagnostic method and the skill with which it is used; and (2) that health is not merely the absence of disease but rather it is a positive quality of life that can be built by degrees to successively higher levels. Thus between the lowest level at which one can live and the much higher level of nutritional well-being to which alone the term *optimal* is properly applied, there is with some nutrients a highly significant field of gradual improvement which we can subdivide more or less elabo-

tely into different degrees or levels or zones, depending in part pon the delicacy of our methods of discrimination and in part upon e nutrient factor with which we are dealing. This last is impornt to emphasize clearly, for it is quite unscientific to assume that e difference between minimal-adequate and optimal intake is ually important for all nutrient factors.

Each nutrient factor should be studied on its own merits in this spect. The difference between minimal-adequate and optimal inke may be less than 10 per cent with calories and more than 100 r cent with calcium or vitamin A or C. And if there is good evince of a very wide difference with one vitamin, this fact creates presumption as to what the difference will prove to be with some her vitamin.

The Concept of Dietary Standards or Allowances in Chemical Terms

During the latter part of the nineteenth century the idea was veloped that the nutritional needs of any animal species could determined with due reference to age, size, and activity; and pressed in terms of the nutrients found by conventional food alysis. Simple arithmetic should then (it was thought) suffice to able the farmer to use his crops or purchased materials in feedg his animals to his best advantage.

For about the space of a generation centering on the turn of the ntury, the same idea was actively developed in relation to huan nutrition. In America this development was largely under the adership of Atwater, who was, however, careful to point out that *dietary standard is only an indication, not a rule.* Atwater also ognized the fact that nutrition can be standardized or brought o equilibrium at different levels.

The National Research Council's Recommended Dietary Allowces aim to furnish guidance for the nourishment of the body at *sirable levels.* These as now conceived by the Food and Nutrition ard of the National Research Council are shown in Table 54.

The term *Recommended Allowances* was advisedly chosen by the

TABLE 54. RECOMMENDED DAILY DIETARY ALLOWANCES [a] REVISED 1948

Food and Nutrition Board, National Research Council

	Calories [b]	Protein, *Gm.*	Calcium, *Gm.*	Iron, *Mg.*	Vitamin A, [c] *I.U.*	Thiamine, [d] *Mg.*	Riboflavin, [d] *Mg.*	Niacin (Nicotinic acid), [d] *Mg.*	Ascorbic acid, *Mg.*	Vitamin D, *I.U.*
Man (154 lb., 70 kg.)										
Sedentary	2400	70	1.0	12 [e]	5000	1.2	1.8	12	75	[f]
Physically active	3000	70	1.0	12 [e]	5000	1.5	1.8	15	75	[f]
With heavy work	4500	70	1.0	12 [e]	5000	1.8	1.8	18	75	[f]
Woman (123 lb., 56 kg.)										
Sedentary	2000	60	1.0	12	5000	1.0	1.5	10	70	[f]
Moderately active	2400	60	1.0	12	5000	1.2	1.5	12	70	[f]
Very active	3000	60	1.0	12	5000	1.5	1.5	15	70	[f]
Pregnancy (latter half)	2400 [g]	85	1.5	15	6000	1.5	2.5	15	100	400
Lactation	3000	100	2.0	15	8000	1.5	3.0	15	150	400
Children up to 12 yrs. [h]										
Under 1 yr. [i]	110/2.2 lb. (1 kg.)	3.5/2.2 lb. (1 kg.)	1.0	6	1500	0.4	0.6	4	30	400
1–3 yrs. (27 lb., 12 kg.)	1200	40	1.0	7	2000	0.6	0.9	6	35	400
4–6 yrs. (42 lb., 19 kg.)	1600	50	1.0	8	2500	0.8	1.2	8	50	400
7–9 yrs. (58 lb., 26 kg.)	2000	60	1.0	10	3500	1.0	1.5	10	60	400
10–12 yrs. (78 lb., 35 kg.).	2500	70	1.2	12	4500	1.2	1.8	12	75	400
Children over 12 yrs. [h]										
Girls, 13–15 yrs. (108 lb., 49 kg.)	2600	80	1.3	15	5000	1.3	2.0	13	80	400
16–20 yrs. (122 lb., 55 kg.)	2400	75	1.0	15	5000	1.2	1.8	12	80	400
Boys, 13–15 yrs. (108 lb., 49 kg.)	3200	85	1.4	15	5000	1.5	2.0	15	90	400
16–20 yrs. (141 lb., 64 kg.)	3800	100	1.4	15	6000	1.7	2.5	17	100	400

[a] Objectives toward which to aim in planning practical dietaries: The recommended allowances can be attained with a good variety of common foods which will also provide other minerals and vitamins for which requirements are less well known.

specific needs. The calorie values in the table are therefore not applicable to all individuals but rather represent group averages. The proper calorie allowance is that which over an extended period will maintain body weight or rate of growth at the level most conducive to well-being.

[c] The allowance depends on the relative amounts of vitamin A and carotene. The allowances of the table are based on the premise that approximately two thirds of the vitamin A value of the average diet in this country is contributed by carotene and that carotene has half or less than half the value of vitamin A.

[d] For adults (except pregnant and lactating women) receiving diets supplying 2000 Calories or less, such as reducing diets, the allowances of thiamine and niacin may be 1 mg. and 10 mg. respectively. The fact that figures are given for different calorie levels for thiamine and niacin does not imply that we can estimate the requirement of these factors within 500 Calories, but they are added merely for simplicity of calculation. In the present revision, riboflavin allowances are based on body weight rather than caloric levels. Other members of the B complex also are required, though no values can be given. Foods supplying adequate thiamine, riboflavin, and niacin will tend to supply

[e] There is evidence that the male adult needs relatively little iron. The need will usually be provided for if the diet is satisfactory in other respects.

[f] The need for supplemental vitamin D by vigorous adults leading a normal life seems to be minimum. For persons working at night and for nuns and others whose habits shield them from the sunlight, as well as for elderly persons, the ingestion of small amounts of vitamin D is desirable.

[g] During the latter part of pregnancy the calorie allowance should increase to approximately 20 per cent above the preceding level. The value of 2400 Calories represents the allowance for pregnant, sedentary women.

[h] Allowances for children are based on the needs for the middle year in each group (as 2, 5, 8, etc.) and are for moderate activity and for average weight at the middle year of the age group.

[i] Needs for infants increase from month to month with size and activity. The amounts given are for approximately 6 to 8 months. The dietary requirements for some of the nutrients such as protein and calcium are less if derived largely from human milk.

Further recommendations:

Fat. There is available little information concerning the human requirement for fat. Fat allowances must be based at present more on food habits than on physiological requirements. While a requirement for certain unsaturated fatty acids (the linoleic and arachidonic acids of natural fats) has been amply demonstrated with experimental animals, the human need for these fatty acids is not known. In spite of paucity of information on this subject there are several factors which make it desirable (1) that fat be included in the diet to the extent of at least 20 to 25 per cent of the total calories and (2) that the fat intake include essential unsaturated fatty acids to the extent of at least 1 per cent of the total calories. At higher levels of energy expenditure, e.g., for a very active person consuming 4500 Calories and for children and for adolescent persons, it is desirable that 30 to 35 per cent of the total calories be derived from fat. Since foodstuffs such as meat, milk, cheese, nuts, etc., contribute fat to the diet, it is necessary to use separated or "visible" fats such as butter, oleomargarine, lard, or shortenings to supply only one third to one half the amounts indicated.

TABLE 54 (*Continued*). RECOMMENDED DAILY DIETARY ALLOWANCES[a] REVISED 1948

Food and Nutrition Board, National Research Council

Iodine. The requirement for iodine is small, probably about 0.002 to 0.004 mg. daily for each kilogram of body weight, or a total of 0.15 to 0.30 mg. daily for the adult. This need is met by the regular use of iodized salt; its use is especially important in *adolescence* and *pregnancy*.

Water. A suitable allowance of water for adults is 2.5 liters daily in most instances. An ordinary standard for diverse persons is one milliliter for each calorie of food. Most of this quantity is contained in prepared foods. At work or in hot weather, requirements may reach 5 to 13 liters daily. Water should be allowed *ad libitum*, since sensations of thirst usually serve as adequate guides to intake except for infants and sick persons.

Salt. The needs for salt and for water are closely interrelated. A liberal allowance of sodium chloride for the adult is 5 grams daily, except for some persons who sweat profusely. The average normal intake of salt is 10 to 15 grams daily, an amount which meets the salt requirements for a water intake up to 4 liters daily. When sweating is excessive, one additional gram of salt should be consumed for each liter of water in excess of 4 liters daily. With heavy work or in hot climates 20 to 30 grams daily may be consumed with meals and in drinking water. Even then, most persons do not need more salt than usually occurs in prepared foods. It has been shown that after acclimatization persons produce sweat that contains only about 0.5 gram to the liter in contrast with a content of 2 to 3 grams for sweat of the unacclimatized person. Consequently after acclimatization, need for increase of salt beyond that of ordinary food disappears.

Phosphorus. Available evidence indicates that the phosphorus allowances should be at least equal to those for calcium in the diets of children and of women during the latter part of pregnancy and during lactation. In the case of other adults the phosphorus allowances should be approximately 1.5 times those for calcium. In general it is safe to assume that if the calcium and protein needs are met through common foods, the phosphorus requirement also will be covered, because the common foods richest in calcium and protein are also the best sources of phosphorus.

Copper. The requirement for copper for adults is about 1 to 2 mg. daily. Infants and children require approximately 0.05 mg. for each kilogram of body weight. The requirement for copper is approximately one tenth that for iron. A good diet normally will supply sufficient copper.

Vitamin K. The requirement for vitamin K usually is satisfied by any good diet except for the infant in utero and for the first few days after birth. Supplemental vitamin K is recommended during the last month of pregnancy. When it has not been given in this manner, it is recommended for the mother preceding delivery or for the baby immediately after birth.

Folic Acid. Evidence for recognizing folic acid (pteroylglutamic acid, vitamin B_c, L. casei factor or vitamin M) as an essential human nutrient is presented in the text. The quantitative requirement cannot be closely estimated from evidence now available.

ood and Nutrition Board of the National Research Council after areful consideration of all suggested alternatives. The Council xplained in its 1948 report that the term "Recommended Allownces" rather than "Standards" was adopted by the Board to avoid ny implication of finality or of minimal or optimal requirements. The quantitative data in the table of Recommended Allowances 're "intended to represent exactly what is implied in a literal interpretation of the words *recommended dietary allowances.*" Hence, contrast to some previously promulgated "standards" or "requirements," the data in the accompanying table are rather to be understood as representing levels of nutrient intakes which the Food nd Nutrition Board recommends as normally desirable goals or bjectives.

The recommendations are not called "requirements" because they re intended to represent not merely the literal (minimal) requirements of average individuals, but "levels enough higher to cover bstantially all individual variations in the requirements of normal eople. . . ."

"Studies on man, as well as more complete experience with animals, clearly indicate substantial improvements in growth and function when the intakes of certain nutrients are increased above the vel which is just sufficient to prevent obvious deficiency symptms. . . . The allowance of a margin of intake above the critical vel for each nutrient is, therefore, designed to permit of additional nefits as well as to cover individual variations. . . . There is now uch evidence from long-term animal experimentation that, aside om individual variations of need, the margins between optimal take and minimal requirements are wider for some nutrients than r others. In the judgment of the Board substantially lower levels an those given in Table 54 would not be expected to give equally od results with large numbers of people through long periods of me. . . ."

Moreover, nutrients may vary widely in respect to desirability of rplus intake. "The usual undiscriminating view is that, if the diet rnishes enough of any given factor to meet functional need, any rther amount supplied by the food is normally a matter very

nearly of indifference, significant only as a sort of insurance agains some emergency. This may be true for most of the essential food factors. Of a few nutrients, however, there is evidence from long term (animal) experimentation that one may, in the course of a lifetime, derive increased benefit from increased intake up to level very considerably above those of ordinarily accepted adequacy (Ascorbic acid, vitamin A, and calcium are perhaps the best established cases of this kind.) Conversely, it may be true of some other nutrient factors that surplus intakes should be held within bounds if undesirable consequences are to be avoided. The outstanding and undisputed example of the latter is the energy value or calories of the diet, of which any considerable surplus intake tends to induce overweight."

For people having ample food at their disposal, the principle of adequacy of energy intake is simple: One may either count the calories or watch the body weight, and eat just liberally enough to have the body weight one wants. Normally the best degree of fatness to maintain is about that which experience has shown to give the best results in the long run. Such long-time experience has been carefully studied and concisely summarized by life-insurance companies and the United States Public Health Service. Their extensive studies show that the average weight for a given height continues to increase up till and into middle age, and that the most advantageous degree of fatness—relation of weight to height—is that which corresponds to the average found at the age of 25 years. This relation of weight to height is shown in Table 24 (Chapter 10) *both* for age 25 (the present "ideal") and age 30 (the previous "standard").

Below the age of 25 a majority of our people tend to be underweight and would do well to build themselves up to the weight-for-height shown in this table for age 25. On the other hand, as they enter middle age most people should be on guard that they do not let their body weight run too much above the new recommendation as indicated in Table 24 in Chapter 10 for age 25 (instead of age 30 as was formerly recommended).

Underweight while perhaps less common and less dangerous (in is country) than overweight, is a real problem with some young omen. Dr. Blunt remarked upon the gain in efficiency and good irits when underweight college women built their body-weights to the life insurance companies' standards for their heights and age 25 or 30.

Energy allowances for children may call for adjustment somehat above or below the Recommended Allowances according to e size, build, and activity of the child. The ranges shown in ble 55 will probably cover ordinary cases.

BLE 55. FOOD ALLOWANCES FOR CHILDREN OF ABOUT 'ERAGE WEIGHT FOR THEIR AGE (Based on Gillett's *Food owances for Healthy Children* and Rose's *Laboratory Handbook for tetics*)

AGE	CALORIES PER DAY	
Years	Boys	Girls
1	900–1200	800–1200
2	1100–1300	1000–1250
3	1100–1400	1050–1350
4	1200–1500	1150–1450
5	1300–1600	1200–1500
6	1500–1900	1450–1800
7	1600–2100	1500–1900
8	1700–2300	1600–2200
9	1900–2500	1800–2500
10	2100–2700	1900–2600
11	2100–2800	2000–2800
12	2300–3000	2100–3000
13	2500–3500	2300–3400
14	2600–3800	2400–3000
15	2700–4000	2400–2800
16	2700–4000	2250–2800
17	2800–4000	2250–2800

The late Dr. Mary S. Rose developed a system of *dietary distribution standards* for children which employs the principle of dicating what percentage of the total calories is to come from each ief kind or type of food. Table 56 shows such standards for chilen from 4 to 12 years of age, arranged in five age groups.

TABLE 56. DISTRIBUTION OF CALORIES IN DIETS OF CHILDREN OF 4 TO 12 YEARS

AGE	PER CENT OF TOTAL CALORIES FROM EACH CLASS OF FOOD					
Years	Foods from Cereal Grains	Milk	Vegetables and Fruits	Fats [a]	Sugars and Sweets	Eggs, Cheese Meat and Other Flesh Foods
4–5	23–25	45–50	14–18	5–8	2–5	5–6
5–6	23–25	45–50	14–18	5–8	2–5	5–6
6–7	20–25	40–45	14–15	10–12	3–4	4–5
8–9	20–25	38–42	15–16	12–13	4–6	5–6
10–12	20–25	34–38	17–18	13–14	6–8	7–8

[a] At least part of the fat to be butter or something known to furnish its equivalent of vitamin A.

Table 57 shows corresponding distribution standards to guide the planning of minimum cost and moderate cost dietaries, respectively for children of ages 5 to 16 years. The minimum cost standard is based on the recommendations of the Committee on Economic Standards of the New York Nutrition Council. The moderate cost standards are those used by Rose and Gray in judging the dietaries of child-caring institutions.

TABLE 57. DISTRIBUTION OF CALORIES IN DIETS OF MINIMUM AND OF MODERATE COST FOR CHILDREN OF AGES 5 TO 16 YEARS

FOOD GROUP	MINIMUM COST DIET	MODERATE COST DIET
	Per Cent of Total Calories	*Per Cent of Total Calories*
I. Foods from cereal grains	37	24
II. Milk	22	32
III. Vegetables and fruits		
A. Dried legumes	3	1
B. Other vegetables and fruits	13	16
IV. Fats and oils	14	12
V. Sugars and sweets	5	7
VI. Meat, eggs, cheese	6	8

Desirability of Two Types of Standards or Allowances

The suggestion has been made by Simmonds and endorsed editorially by the *Journal of the American Medical Association*, that

the present stage of development and dissemination of nutritional owledge there is "too much of a tendency" to discuss nutrition in ms of the individual chemical factors involved, and that there is ore to be gained" through discussion of balanced nutrition in ms of the articles of food which one actually chooses and con- nes. Undoubtedly we should use both these plans.

The U. S. Department of Agriculture's *Family Food Plans* are strations of guidance to dietary adequacy which is based on ormation of both types but is given in terms of everyday articles foods. The grouping of foods which makes it possible to give nu- ional guidance in this non-technical way, yet through it applying best scientific knowledge, is discussed more fully in what ows.

The Problem of Factors as Yet Unmeasured and the Principle of Natural and Nutritional Wholes

as our present list of nutritionally essential factors may not yet qualitatively complete, and certainly the quantitative needs for ne individual factors have not yet been measured with perma- tly satisfactory precision, it is helpful to consider the general nciple of *natural wholes.** An organism is more than a mere mation of its parts, it is an *organized* whole and functions as h in nature. Many of nature's wholes have evolved under the uence of each other, and so bear relationships to each other ch have been more or less fixed by the evolutionary process. s doubtless true that if we tried to subsist upon mixtures of the wn chemical essentials in artificially purified forms we might ner or later find ourselves inadequately nourished for lack of ething still unknown. Yet it is also true that if instead of arti- lly purified foodstuffs we use natural foods such as have con- ited a part of the environment in which our bodies have evolved to which they are nutritionally adjusted, then any essentials

* The principle of natural wholes has been developed philosophically by ts under the term "holism" in his book, *Holism and Evolution*, and in an le on holism in the *Encyclopaedia Britannica*.

which may be still unknown are consumed as they occur in the natural wholes which we eat.

Closely related to the principle of natural wholes is that of nutritional flexibility.

The Principle of Nutritional Flexibility

Not only is the body an organized natural whole which is something more than a mere summation, but also *as organism* it is much more than a machine.

One who wishes may call the metabolic process "mechanistic" in the sense that we formulate our concepts of it in physico-chemical terms which do not depend upon the assumption of any wholly mysterious vitalism. Yet, even so, this concept should incorporate the fact that the body possesses an important degree of flexibility in its nutritional processes.

By virtue of this the body can make nutritional adjustments, and so we can make good use of somewhat fluctuating food supplies.

This principle of nutritional flexibility is only in part a matter of simply raising or lowering the level of metabolism of a given factor, though at times this may be very important. There are also many cases of what is called sparing of one nutrient by another; and such cases are not all of any one chemical category. Three illustrations of nutritional flexibility follow:

(1) To some extent the body can adjust itself to a lowered total food intake by lowering its rate of basal energy metabolism, as well as by a loss of weight which reduces the expenditure of energy involved in bodily activities, external and internal. Benedict found that healthy young men could reduce their daily food calories by about one third, partly by loss of weight and partly by lowered basal metabolism. And in these cases the subjects continued their accustomed mental work and physical exercise and experienced little adverse effect beyond a diminution of animal spirits. Under severe wartime food restrictions, there have appeared to be much more drastic reductions of energy metabolism; but how far these

ve been physiological adjustments and how far pathological is not finitely known.

(2) By liberal intakes of carbohydrates and fats so as to make ceptional use of their protein-sparing powers, the rate of protein etabolism may be reduced to 30 grams a day or less in healthy, rmally active men. This fact has been familiar for so long that its gnificance as an example of flexibility in nutrition is perhaps com- nly overlooked.

(3) A recent example among vitamins is the finding that ribo- vin can in some sense "spare" thiamine as shown by the fact that ts from families on high riboflavin diet show increased ability to dure shortage of thiamine.

These three examples are chemically so different as to indicate at the general principle of nutritional flexibility is of wide appli- tion.

By means of feeding or injection experiments with nutrients (or mpounds of nutritionally essential elements) containing "tagged" oms, it has been possible to show in easily impressive ways much at previously could be grasped only through more intricate knowl- ge, as to how the body manages its nutritional resources. See, r example, the monograph entitled, "The Dynamic State of Body nstituents," * and many papers in the *Journal of Biological Chem- ry* and elsewhere, several of which are included among the read- gs suggested at the ends of this and previous chapters. The trend this relatively recent development is to emphasize strongly the ct that nutrient material received by the body is distributed omptly and widely to the various organs and tissues where it may transformed in either one way or another. Flexibility is thus a ore important aspect of nutrition than previously appreciated. t as was carefully pointed out as early as 1939,† the isotopes of element are not quite identical in chemical properties; and this especially true of the elements of relatively low atomic weight

* Begun by the late Dr. R. Schoenheimer, finished by Professor H. T. Clarke, d published by the Harvard University Press (1942).

† By T. Ivan Taylor, *Science* **89**, 176–177 (February 24, 1939).

which in general include those chiefly concerned in nutritional processes. Hence the indications obtained from biochemical experiments with isotopic tracers should be investigated further by long-term feeding experiments with natural foodstuffs.

On the practical side, this nutritional flexibility means, among other things, that with a careful, continuing translation of our laboratory findings into terms of food commodities it is possible now to use our total food resources, or a so-called surplus of any of them, in such flexible but scientifically guided ways as to make them function more effectively for widespread nutritional well-being than we formerly knew how.

Non-Technical Guidance Toward Adequate Nutrition for All: Proposed National Food Allotment Plan

In 1944 hearings were begun in the U. S. Senate Committee on Agriculture upon a bill (S–1331) to provide a *National Food Allotment Plan* with the purpose of bringing within reach of low-income families the equivalent of the following foods per person per week.

TABLE 58. PROPOSED WEEKLY ALLOTMENT (see text)

Milk, 5 quarts
Potatoes and sweetpotatoes, 4 pounds
Dry beans, peas, and nuts, 8 ounces
Tomatoes and citrus fruits, 1½ pounds
Green and yellow vegetables, 1½ pounds
Other vegetables and fruits, 2 pounds, 5 ounces
Eggs, 4 (number of eggs)
Meat, poultry, and fish, 1½ pounds
Flour and cereals, 4 pounds, 7 ounces
Fats and oils, 14 ounces
Sugars, sirups, and preserves, 12 ounces

The fact that any proposal of this kind should receive such serious consideration is significant of the national awakening to the importance and practicability of eradicating malnutrition.

There is need to clarify the meaning of malnutrition and the facts as to its prevalence.

Harmonizing the Criteria of Dietary Adequacy and Nutritional Status

The testimony of sufficiently nutritionally-minded physicians ows that the actual prevalence of malnutrition in the United States much greater than is indicated either by Federal and State sta- tics of morbidity and mortality, or by hospital records. Probably is also true that malnutrition is more common than most practic- g physicians are yet aware. The reasons that so much of the mal- trition goes unrecognized are several: The more delicate methods r the diagnosis of the different nutritional deficiencies have been veloped only so recently that relatively few physicians have the vanced knowledge and equipment required to make comprehen- e, up-to-date examinations in this field of medicine; so the larger mber of cases of malnutrition are not seen by anyone in position recognize them and are reported according to such symptoms as int to some more commonly recognized disease. And also there e traditional priorities in the classifications of patients and of uses of deaths, so that even when the attending physician does port a nutritional deficiency the supervisory hospital official or e registrar of deaths will still probably classify the illness or death essentially a case of some disease more commonly recognized an are most types of malnutrition.

On the other hand, when the kinds and amounts of foods con- med by a fair sample of American families are computed to their trient values and compared with the National Research Council's ecommended Allowances, an appreciable proportion of these etaries fall short of this standard in respect to one or more of eir nutrient values. Some critics have held that either the dietary servations must be at fault or the Recommended Allowances must nstitute too high a standard by which to judge adequacy, inas- uch as practicing physicians do not find nearly so many cases of alnutrition as the dietary studies would seem to imply.

It is true that the Recommended Allowances aim at adequacy r *best results* and not merely for prevention of disease. Also, in the

use of such an eight- or nine-point * "yardstick" there is an increased chance that an accidental fluctuation in the food supply might make an apparent shortage at some one point which in practice might be fully compensated within the next few days. Hence we should be careful to distinguish between the nutritional adequacy of the dietary, on the one hand, and the nutritional status of the body on the other. (Thus we might say that a man is ill-fed any day that he does not get a nutritionally good dietary; but that only after a considerable period of ill-feeding does he become a malnourished man.)

If, as now appears, we may consider that when the best methods of study are used there is reasonable harmony between the findings of dietary studies and the surveys of nutritional status, it is well for us also to realize that the two modes of enquiry do not measure quite the same thing, and that precise parallelism is not to be expected. In general, too, it is to be expected that an increase or decrease of nutrient intake will influence the level of concentration of the nutrient in the body's tissues and fluids more quickly than it will influence the structural tissue changes seen in biomicroscopy.

Thus there may not be complete and precise accord even as between the more chemical and the more physical of the delicate diagnostic methods. But neither as between these two types of methods of studying bodily status, nor as between either of them and a careful dietary survey, is there occasion for serious lack of confidence in the essential significance of the new knowledge.

The *total* food consumption in the United States is such as to feed all its people in accordance with the National Research Council's Recommended Allowances. The *distribution* of the nation's food among its people is, however, uneven; and many low-income families are ill-fed as shown by dietary studies, and show a high prevalence of malnutrition in medical surveys of nutritional status. The problem of eradicating this malnutrition is discussed in later chapters.

* While ten factors were included in the table of Recommended Allowances, we consider that certainly with vitamin D and probably also with niacin (as explained in Chapter 20) it is misleading to compare figures in the table of allowances with results of analyses of foods.

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Chapter 29

CONSCIOUS CHEMICAL CONTROL OF THE INTERNAL ENVIRONMENT: THE PROBLEM OF THE BEST USE OF FOOD

The Concept of the Internal Environment or Fluid Matrix of the Body

Ordinarily we consider ourselves as air-inhabiting animals; but our living substance is separated from the air by a layer of relatively inert material—the skin and the mucous membranes on our outer and inner surfaces, respectively. As Cannon * put it, except on their sides where they are contiguous one with another, the cells of our bodies are in contact with a watery environment or *fluid matrix.* The blood consists of immense numbers of red cells or corpuscles (*erythrocytes*), and also many white corpuscles (*leucocytes*), both kinds of corpuscles floating in a thick watery solution and colloidal dispersion, the *plasma.* Because blood and lymph are limited in amount, the only way in which they can function fully and continuously as carriers is by being circulated in the body.

Influence of Food Selection upon Internal Environment

For nearly a century, science regarded the *internal chemistry* of the normal members of each species as being more rigidly specific than it really is.

As briefly mentioned in previous chapters, Liebig's investigations seemed to show that a plant or an animal will grow only so fast and so far as is consistent with the attainment and maintenance of the specific chemical composition of its kind.

* W. B. Cannon 1939 *The Wisdom of the Body,* Revised Ed. (New York: Norton.)

Somewhat later than Liebig, another great scientist and influentia teacher, Claude Bernard, gave cogent expression to essentially th same view in its more physiological aspect when he said that it is th *fixité* of the internal environment which enables an organism t cope with a new or changing external environment. The neat form of this generalization, and its usefulness in helping to explain the self-regulatory processes in the body and the spread of species over the surface of the earth, have resulted in its being accepted and repeated somewhat dogmatically for the past two or three generations.

Meanwhile, however, there have developed *physico-chemical principles*, based on much more complete evidence, which call for a thorough revision of these over-simple postulates or first-approximations. We can no longer doubt that when different proportions of active factors are introduced into a system, some difference in concentration levels or dynamic-equilibrium points must result. The postulated *fixité* of the body's internal environment can therefore be only approximately true; and, in its chemical aspects, must vary with the individual choice and use of foods. We normal people living on everyday foods introduce into our systems different amounts and proportions of such active factors as are some of the mineral elements and vitamins, depending upon our choices among the staple foods and the relative proportions in which we consume them.

Until recently it could be thought (and doubtless it is still thought by many people who have not sufficiently studied the most recent evidence) that while what has just been said of concentration levels and equilibrium points is theoretically true yet practically the body's physiological regulation keeps its internal chemistry essentially constant. But, as has been seen, analyses of blood plasma and of cerebrospinal fluid show that their concentration levels are significantly influenced by the levels of nutritional intake, even in the case of so soluble and diffusible a substance as vitamin C. Also, analyses of the body as a whole show (Chapters 14 and 27) that the calcium content of experimental animals, and doubtless also of human beings, is significantly influenced by the calcium content

of the food. And this is true not only as between adequate and inadequate intakes, but also as between different degrees of liberality of intake within the normal zone.

As we have seen in previous chapters, vitamins A and C, calcium, and riboflavin, each has shown added favorable response to added liberality of intake up to levels much higher than those of merely passable adequacy.

It may be recalled, as well, that by calcium balance experiments with children, it has been established that the rate of calcification in the normally developing body, and therefore the percentage of calcium in the body at a given age or size, may be varied by adjustment of the nutritional intake of calcium at a higher or a lower normal level, and that there are strong clinical indications that the higher normal levels lead to better human life-histories. And more comprehensive methods of controlled experimentation have now also been developed.

Full-life, successive-generation experiments with large numbers of laboratory animals (of species whose nutritional responses have been shown to be like those of human beings with respect to the nutrients under investigation) indicate that, in addition to calcium, riboflavin (Chapter 19) and vitamin A (Chapter 23) also show similar wide zones between the minimal-adequate and the optimal levels of intake. This has also been found true of vitamin C (Chapter 17), insofar as long-term experiments at different intake levels have been tried.

That riboflavin and vitamin C are water-soluble, vitamin A a fat-soluble, and calcium in the form of bone mineral a relatively insoluble substance, shows that the principle of the flexibility of the body's internal environment and of its control through levels of nutritional intake is applicable to substances of a wide range of properties.

While, in the cases just considered, the optimal levels of intake are relatively high, it is also to be noted that some other substances or elements may be essential factors in our nutrition and yet their optimal levels may be in the lower or middle, rather than the higher, ranges of the normal zone. For calcium, riboflavin, and vita-

min A are not "random samples" of nutrients; they were submitted to the long investigations briefly mentioned here because of the probability that among them might be found a chemical explanation of earlier nutritional research data in which the experimental variable was the quantitative relation between natural articles of food in the dietary.

The fact that in each of the above-cited cases the zone between the merely adequate (minimum-adequate) and the optimal level was found to be strikingly wide is therefore not to be taken as indicating that correspondingly large intakes of other nutrients will be correspondingly beneficial. For while we recognize, for example, chloride ions and cholesterol as having essential functions in our nutritional processes we do not believe that our bodies would be benefited by being kept saturated with chlorides and cholesterol, nor by larger intakes of *these* nutrients than we usually get in a chance-chosen dietary.

As yet most of the nutritionally essential mineral elements, amino acids, and vitamins have not been investigated by full-life experiments to find out precisely what degree of liberality of intake gives best long-run results. And we cannot scientifically infer this *from* any one nutrient *to* any other one. Each must be studied independently on its own merits.

Hence the lesson to be learned from our new knowledge of the internal environment is not a doctrine of indiscriminate liberality in the consumption of all of the essential nutrients; but rather it is a principle of scientific discrimination. The real test is whether (and, if so, how) a difference of intake influences the outcome of full-life and successive-generation experiments such as have been developed in only the most recent years and as yet in relatively few laboratories. Such long-time feeding experiments in large numbers and well controlled, may be valid even in cases in which we have not yet devised analytical methods sufficiently delicate to measure the difference of concentration in the body that is produced by a given difference in the level of nutritional intake.

Thus Stiebeling has written that food plays an important part in determining the internal environment, and that differences in

this environment, many of which may be too small to be measured by present *in vitro* methods, "definitely affect the plane on which physical and mental functioning go on."

And Stare, speaking both as biochemist and physician, has strongly emphasized the importance of the fact that "the internal environment of the body, the 'milieu interne' of Claude Bernard, is not a fixed environment, but can be readily changed by the choice of *food.*" (The italics are Dr. Stare's.)

Experiments Planned in Terms of Whole Lives

From the viewpoint of this strong emphasis upon the influence of food on the internal environment, one might answer the question suggested in the title of this chapter by saying that the best use of food is that which results in the best internal environment of body. That is entirely true so far as it goes. How, then, is one to know when the internal environment is at its best?

Experimentally this is best ascertained by feeding the diets one wishes to compare to parallel families of laboratory animals (of like heredity and kept in like surroundings), throughout the entire lives of successive generations, with systematic recording of objective criteria of nutritional well-being throughout. With animals of short natural life cycle one can have a comprehensiveness and exactness of control that is not to be expected with human subjects. In human experience so many factors may enter to influence health in the course of a lifetime that it would under the best conceivable conditions be hard to separate and measure the effects of food alone upon the whole duration and efficiency of life. But this can be, and has been, done with laboratory animals of rapid growth and early maturity, such as the rat, and it has been possible to determine under conditions uniform in all other respects, the influence of modifications of the diet upon the various criteria of health. And as we see here and elsewhere, among the findings of nutrition experiments, carried through successive generations of such laboratory animals, is the fact that starting with a dietary already adequate according to current standards, we may, by improvement of the diet, induce

a higher degree of health and vigor, and a longer and better life history.

The fact that the rat, while resembling us so closely in most aspects of its chemistry, yet runs through its normal life cycle in one thirtieth the time of a human life, adds very greatly to the facility with which this deputy can be employed in the experimental study of our nutritional problems. If it were possible to observe human subjects under controlled conditions through entire life cycles for successive generations, centuries would be required to cover with man the ground covered by the laboratory work with rats that was devoted to the comparison of Diets A and B, described in Chapters 14 and 34.

The increase in average length of adult life here found would correspond to an extension of the longstanding human-adult life-expectation of 70 years to 77 years instead. Obviously many cases of people living to the age of 77 fall within the familiar range of normal experience. Yet inasmuch as previous improvements in the average length of life had been so closely confined to the lowering of *early* death rates as to leave the average length of *adult* life unchanged, the possibility of extending this adult average by a better use of food is of great interest, and of real value as conservation of our human resources.

It is all the more significant because of the fact that, in these experiments, development is expedited and senility deferred in the same individuals, so that what, for lack of a better term, we may call "the period of the prime" is extended in greater ratio than the life-cycle itself.

And, so far as we can judge, the positive improvement of health, the building of a higher and more buoyant health, can be added to the gains in health, efficiency, and longevity which are attained in other ways, as through genetics, sanitation of environment, and training of mind and body.

The experiments here described were chemical and biological—not psychological—but so far as our minds are domiciled in our bodies, it seems reasonable to expect that a superior internal environment may have a more than merely biological value.

At any rate, we may now accept as established, (1) the existence of a wide and evidently fruitful area of research between the merely adequate and the optimal in food supply and nutritional condition, and (2) the general validity of our present methods of animal experimentation in this field of research.

It should be emphasized that a given improvement in use of food does not always influence all segments of the life cycle in comparable fashion as in the case of Diets A and B described in Chapters 14 and 34. In that case the rate of growth and the length of life were both increased. But under very different circumstances or by some other kind of change in regime, what makes growth more rapid may sometimes shorten and sometimes lengthen life.

The average of a sufficiently large number of life histories of experimental animals, of well-chosen species with ample controls, may be made a more delicate means of finding a difference than are current methods of chemical analysis. For example, the bone trabeculae (Chapter 14) keep the calcium content of the blood so nearly constant that chemical analysis can only doubtfully reveal the day-to-day differences.

Yet we now know that differences of intake levels of calcium may influence the life history through the blood chemistry, even though the findings of direct chemical analyses *in vitro* may be still inconclusive.

Pros and Cons of High Nutrient Intake

Researches upon relations of nutrition to longevity, carried on independently and simultaneously in the laboratory of animal nutrition at Cornell and the laboratory of nutritional chemistry at Columbia, have yielded certain results which at first glance may be confusing, in that at Cornell longer life followed retarded growth, while at Columbia increases of growth rate and length of life both resulted from the same improvement of the food supply. Yet the Cornell and Columbia findings are in harmony when the great difference in the dietary starting-points of the two researches is considered. And the consideration of this difference not only clarifies

the interpretation of these particular experiments but also gives a hint of the breadth of significance for human progress, in the understanding and utilization of material resources, which this general field of research now offers.

The dietary starting point of the Cornell experiments was a food mixture which had been made extraordinarily rich in proteins and vitamins so that the intakes of these nutrient factors would still be liberal when the daily allowance of the food mixture was so reduced as to control the rate of growth through the regulation of the food calorie supply. The extreme richness (with palatability) of this food supply, per calorie, was such as may be approached in occasional cases of the "forcing" of farm animals for maximal gains in body weight; and possibly when an infant is fed without regard to economic considerations and with too great a desire to make a phenomenal growth record. In the nutrition of farm animals the problem of the advisability of "forcing the growth" by *ad libitum* feeding of extremely rich food mixtures may frequently be of economic importance. In human nutrition such problems would arise if at all, only in the homes of the small minority of families of highest income, or in the minds of pediatricians practicing in correspondingly privileged groups. In contrast, the dietary starting point of the Columbia experiments was representative of the food supplies upon which most people live, with only an economical proportion of protective food.

Thus, scientific examination of the above-mentioned Cornell and Columbia findings shows them to be quite consistent when the differences in the plans and starting-points of the respective experiments are considered. If the findings look different at first, it is because they are the results of cultivation of the opposite edges of a very wide field of research. In fact, exploratory work has already shown that different nutritional improvements may differ as to the part of the life process upon which they confer their chief benefits. Thus in certain circumstances where increased vitamin A value of the family dietary had no apparent further effect upon growth or reproduction it did decrease the adult death rates and increase the length of life.

In order to learn how to give our life processes an optimal chemical environment in which to develop and function, we shall doubtless need to treat our bodies with somewhat more intelligence than merely to feed into them "plenty of everything." For we now know that there are very important differences among the nutrients with respect to whether surplus intake: (*a*) is added to the body's actively working capital; or (*b*) functions as a passive reserve or insurance; or (*c*) whether anything beyond a very moderate surplus of the particular substance would best be avoided.

Moreover, in addition to these differences of status or surplus of an individual nutrient considered by itself, there are considerations of balance or imbalance in our intakes of relative amounts of some two or more of the forty-odd nutritionally essential substances for which we depend upon our food (and drinking water).

Groupings of Foods as Guides in Their Use

Simple arrangements of foods into five, seven, ten, or eleven groups have each been found helpful in certain circumstances.

A five-fold grouping, which may be a first step toward a balanced food budget and improved internal environment, is as follows:

Divide your food money into fifths—

One fifth, more or less, for vegetables and fruit;

One fifth, or more, for milk and cheese;

One fifth, or less, for meats, fish, and eggs;

One fifth, more or less, for bread and cereals;

One fifth, or less, for fats, sugar, and other groceries and food adjuncts.

The "Protective Foods" and the "Basic Seven" Food Groups

Relatively early in his epoch-making studies of the nutritional characteristics of the different types of foods, McCollum reached the point of view that the two nutritional shortages most often occurring in American dietaries are those of calcium and vitamin A value; and that the two types of food which are relatively rich in both of

these are milk and the green leaf vegetables. He therefore proposed that these be bracketed under the term "protective foods" to emphasize their special value in protecting us against our most frequent dietary deficiencies.

Further investigation brought a growth of knowledge that broadened this concept in both of its aspects.

With no diminution of emphasis upon calcium and vitamin A value, but with fuller appreciation of vitamin C and with the discovery of the far-reaching importance of riboflavin and the frequency with which it is the "limiting factor" in dietaries, there came about a broadening of the use of the term protective foods to cover fruits, vegetables, and milk, with or without eggs.

Obviously there are fairly wide differences among fruits, and also among vegetables, as to the degree in which each fruit or vegetable deserves the term "protective," yet considering McCollum's term (as he intended it to be) merely as a device for simple non-technical teaching and as an aid to memory, the broadening of its use to include fruits and vegetables generally seemed more helpful than the erection of subdivisions that would doubtless need to be shifted from time to time as knowledge grew. Obviously too, milk as a protective food should include its fresh, canned, and dried forms and also cheese, cream, and ice cream. The place of the egg is less clear. For while the egg, on the one hand, seems to rank as protective by virtue of its relative richness in vitamin A and riboflavin, along with a moderate calcium content, yet on the other hand it differs from milk and from most fruits and vegetables in not contributing to the maintenance of superior intestinal hygiene or of the body's alkaline reserve. There is also the problem whether if eggs are eaten very freely as a habit at all ages, they may bring into the body more cholesterol than may be best.

In addition to the unavoidable ambiguity introduced into the concept of "protective foods" by the special characteristics of eggs and of some of the fruits and vegetables, the usefulness of the term has been further impaired by its having been, in some connections, stretched out of due resemblance to the original concept. Chiefly for this latter reason, the term protective foods seems now to have

been rendered so ambiguous that our best plan is probably to discontinue its use wherever practicable. In any connection in which it may still occur in this book it may be construed as covering fruits, vegetables, and milk, with or without eggs.

The "Basic Seven" Food Groups were set up by the Department of Agriculture and the War Food Administration as an aid to the United States Government's widespread non-technical teaching of nutrition and food values. The doctrine is, "Eat every day from each of these Basic Seven Food Groups:" (1) Green and yellow vegetables; (2) Citrus fruits, tomatoes, and salad greens; (3) Other fruits and vegetables; (4) Milk, including all its recognized forms but not butter; (5) Meats, fish, poultry, eggs, nuts and mature legumes, including peanut butter; (6) Breadstuffs and cereals, whole grain, enriched, or restored; (7) Butter and fortified margarine. "After these eat any other (wholesome) food you like."

Clearly this is not the place for a full discussion of this "basic seven" food grouping: for primarily it is designed to serve a type of teaching much more elementary than is undertaken in this book; and while we might discuss the grouping critically from the viewpoint of our present study, this would involve overlapping the subject-matter of some other chapters.

Yet we may well note in this simple seven-fold grouping, a few facts that bear upon the present chapter's problem of the best use of food: A brief sketch of the guiding facts and principles in our problem need not necessarily involve an attempt to evaluate every kind of food we eat. What we know about optimal diet may be stated in terms of its fundamental (or in the non-technical sense "basic") aspects, leaving each reasonably rational individual free, as McCollum so well put it, to eat what he likes so long as he eats what he should. And the statement of what we "should" eat may be fairly elastic and still be in full accord with our modern knowledge, because, as explained in Chapter 28, our nutritional processes are endowed with a considerable degree of flexibility. Governmental guidance addressed to all the people at once very properly seeks to suggest as little change in the food habits which people at present "like" as is consistent with their good nutrition and with good use

of the food supply that the nation is currently accustomed to produce. In contrast, the students of this book will be so small a part of the population that if they go farther in the direction indicated by the newer knowledge of nutrition than the "rule" of the "Basic Seven" calls for, thus making themselves leaders instead of merely followers in the movement for better nutrition, these seekers for the *best* will not be so rapidly growing a part of "the market" as to throw American agriculture out of balance. Good citizenship does not demand that they be inhibited by any such fear, but *does* suggest that those who are qualified by nutritional knowledge *should* be leaders, both by example and precept in a *continuing* advance in the direction in which the literal following of the basic-seven rule is a first step. This will continue to be true in the scientific search for guidance to the permanently best use of food, even if commercial conditions should lead to some temporary backsliding at any time.

Let us, therefore, recognize the significance of each of the seven food groups that the Government's elementary teaching has characterized as basic and make this a first lesson in our study of use of food. Then proceed to a more discriminating study of foods in terms of ten or eleven groups.

Green and yellow vegetables are bracketed, of course, because of their high vitamin A value; and the second group of the basic seven is a bracketing of foods rich in vitamin C. As explained in the next chapter, there is some overlapping between these two groups of foods, from the viewpoints of both their vitamin A and C values. So important are both these factors in human diets that we seek to make optimal, that the best use of food may well involve the inclusion of more than one serving from one or both of these groups in almost every daily dietary.

The setting-up of a third "basic" group for all other fruits and vegetables is a means of commending these to the appreciation of consumers, and as such is well justified. But as the fruits and vegetables of this group are not outstandingly rich in any characteristic nutrient, their place can be taken by increased consumption of items from other groups; and, when prices are right, such "substitution" might constitute a still better use of food than a literal adherence to the basic-seven rule.

Milk in one or more of its various forms may well be a part of nearly every meal and (for reasons apparent from other chapters) it is quite clear that a decidedly increased prominence of milk in the diet is an important step toward the best use of food, – and also of our national food-production resources.

It is a good feature of the "basic seven" that includes eggs in the same group with meats, fish, and poultry; for when milk in its various forms (including cheese, cream, and ice cream) is used as freely as it should be in a dietary that seeks "best use of food," there is then no need that both egg and meat should also be included in the same day's food. Either an egg or a serving of meat, fish, or poultry will constitute an ample protein enrichment of a well chosen dietary containing a liberal amount of milk. And if "the problem of the best use of food" is construed to include consideration of such use of food resources as shall tend to spread excellent nutrition to as many people as we well can, then on at least one day a week the high-protein dish of the day may well be beans or peas instead of either meat, fish, poultry, or eggs.

No space need be given to comments here upon breadstuffs and cereals, or butter and other fats, in view of what is said in other chapters.

Food commodities as classified in 10 or 11 groups will be discussed in Chapter 30, in the study of which it should be kept in mind that there are differences *within* as well as between some of the food groups.

Flexibility in the Concept of the Body's Steady States

W. B. Cannon's findings and views deserve further consideration for the light which they throw upon the nutritional aspects of our internal environments; because, to a very significant degree, these are what we make them by our decisions as to what foods to eat and how much of each.

As Cannon puts it, the red cells of the blood play a vital role in the body because in the lungs they are able to take on very quickly a practically full load of oxygen and then to unload this oxygen more or less completely in other parts of the body where there are cells in

need of it. Also they carry carbon dioxide from other parts of the body to the lungs for elimination. The white corpuscles serve as scavengers and protectors against invading microorganisms and other foreign particles. The plasma amounts to more than half of the total mass of the blood. It distributes throughout the body "all manner of food materials provided by the final digestive processes in the intestines." But the kinds and amounts of nutrients thus distributed by the blood to the body cells and tissues are, of course, dependent upon the kinds and amounts of foods eaten, as well as upon the rightness of the digestive and distributive processes.

Lymph differs from blood chiefly in containing no red cells and less protein; but does contain white corpuscles, some other forms of protein, glucose, and salts. Much of Cannon's discussion of *homeostasis* (the term he coined to indicate the body's "steady states") has to do with the bodily function of utilizing the irregular amounts of nutrients received from the food and at the same time maintaining a *relative* constancy of concentration of each nutrient in the body's fluid matrix.

The efficiency of this fluid matrix in the maintenance of its water and salt content has been mentioned in Chapter 12. Cannon's discussion of the homeostasis of blood sugar (glucose) is of special interest here as an example of the degree of variation which may exist normally. He states that when the concentration of glucose in the blood rises in response to sugar-rich food until the "kidney threshold" is reached at about 180 "milligrams per cent" glucose passes from the body in the urine. "If on the contrary the concentration falls to 70 milligrams per cent or lower, the 'hypoglycemic reaction' is likely to appear. Thus the normal bounds are here taken to be 70 and 180 milligrams." Hence a low of 70 and a high of 170 to 180 are both within the normal homeostatic zone. Thus the body's justly celebrated "steady states" are only relatively so, and it is easily conceivable that differences within this range might become significant within less than the long run of a lifetime.

We may conclude (1) that one of the most far-reaching advances in the chemistry of nutrition is the finding that the body's internal environment may be importantly influenced by the food, and is thus

amenable to our conscious chemical control; and (2) that natural or induced differences within the normal range may have significant effects in long-term experience.

Hence the chapters which follow may well be read with eyes and minds especially open to practicable applications of the principles involved in our study of the internal environment and its control through our use of food.

If one or more of the following paragraphs seems similar to something you have read before—if there should even be some near-repetition—the reader is asked to give it special thoughtfulness on that account.

The body's internal environment is readily changed chemically by alteration in the amounts or relative proportions of nutrient factors—elements, or molecules, or closely related groups of molecules, as the case may be.

Such a chemical change in the body's internal environment may be too small to be shown by present methods of analytical chemistry and yet it may be found to have important effects when looked-for by adequately controlled quantitative feeding experiments extended throughout entire lifetimes and perhaps successive generations.

Experiments in the chemical laboratories of Columbia University with calcium and with protein are interpreted in this way and have now been completed in sufficient numbers to be regarded as statistically conclusive.

In other experiments of the same general type, with vitamin A as the variable factor, increased intakes *both* increase the body's store of the vitamin and also markedly improve the life history.

From the above and other experiments we now have the generalization, that increased intake of a given nutrient sometimes is, and sometimes is not, accompanied by an analytically demonstrable increase of concentration of such nutrient in the fluids and tissues of the body.

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Chapter 30

NUTRITIONAL CHARACTERISTICS OF THE CHIEF GROUPS OF FOOD

The simplest grouping of foods which is worthy of serious study today is that used in the 1943 Report of the Secretary of Agriculture, as follows:

"Considered in terms of nutrition, foods can be classified in five groups, each of which has a characteristic function. Though they have some overlapping functions, these groupings offer guides to food needs. By combining foods from each of these five groups it is possible to obtain food supplies containing all the essential nutrients. The five groups are:

"1. Grain products: Important as inexpensive sources of energy and protein. The lightly milled products are also excellent sources of iron and vitamins of the B group.

"2. Fats and sugars: Economical sources of food energy. Some of the fats also carry important amounts of vitamin A. These foods give to diets flavor and satiety value which are highly prized in many parts of the world.

"3. Meats, poultry, eggs, fish, legumes, and nuts: Important sources of proteins and certain of the vitamins of the B group. Eggs are sometimes classed separately because of their richness in vitamin A.

"4. Milk and milk products: The outstanding economical source of proteins of high quality, calcium, and riboflavin. Also important for many other vitamin and mineral elements.

"5. Vegetables and fruits: These differ widely in their nutritive value. Special emphasis should be given to the leafy green and yellow vegetables for their vitamin A value; tomatoes and citrus fruits (among others) for vitamin C value; potatoes and other starchy tubers as economical sources of energy and of some of the mineral elements and vitamins."

Classification of Foods into Ten * or Eleven Groups

The eleven food groups, largely used in the planning and evaluation of dietaries and food supplies, make use of the above five major

* In some discussions, eggs are included in the group with lean meat, poultry, and fish.

divisions but subdivide some of them so that with a larger number of groups each may be more nearly homogeneous or closely related from the viewpoint of the dietary or meal planning, or that of the planning and production of the Nation's food supply. Listed in the order that seems most effective for the present discussion, these groups are: (1) Grain products; (2) potatoes and sweetpotatoes; (3) dry mature beans, peas, and nuts; (4) tomatoes and citrus fruits; (5) green and yellow vegetables; (6) other vegetables and fruits; (7) milk and its products other than butter; (8) eggs; (9) lean meat, poultry, and fish; (10) fats, including bacon and salt side; (11) sugars, sirups, molasses, and preserves.

Table 59 shows the relative prominence of each of these eleven food groups in the cost, and as to its contribution of certain nutritional factors, in a U. S. Department of Agriculture study of food consumption in 1942.

In contradistinction from the tabulated *recommendations,* the data in Table 59 summarize the *food actually consumed* by a presumably representative sample of our population.

For each of the eleven groups into which it is now customary to divide a dietary or food supply, Table 59 shows the percentage of each nutrient factor contributed by each food-group, and what percentage of the food-money was expended for the food-group in this study.

From the viewpoint of the title of this chapter, the nutritional character of each food-group may be studied largely by comparing its prominence in the Calorie column with its prominence in the column for each nutrient. From the viewpoint of Chapter 32, chief attention would be paid to the comparison of the cost column with the column for each nutrient factor.

Table 59 thus belongs to both Chapters 30 and 32.

It is always to be remembered that, while some of the groups are fairly homogeneous, there are other cases which present significant differences *within* as well as *between* the food groups. Thus, for example, butter and enriched margarine, which are important carriers of vitamin A, are grouped with other commercial fats and bacon, which contain practically none of this factor.

TABLE 59. RELATIVE COST AND CONTRIBUTIONS TO NUTRITIVE VALUE OF EACH FOOD GROUP: U.S.D.A., STUDY OF SPENDING AND SAVING IN WARTIME, SPRING OF 1942, AMERICAN FAMILY DIETARIES

FOOD GROUP	PER CENT OF TOTAL FOOD COST	PERCENTAGE OF TOTAL CONTRIBUTED BY EACH FOOD GROUP							
		Calories	Protein	Calcium	Iron	Vitamin A Value	Thiamine	Riboflavin	Vitamin C
Grain products [a]	11	30	28	12	21	[b]	22	9	0
Dry beans, peas, nuts	1	3	5	3	11	[b]	6	3	0
Potatoes, sweet-potatoes	3	5	3	2	7	6	8	4	13
Green and yellow vegetables	5	1	2	5	8	39	6	5	31
Citrus fruits, tomatoes	6	2	1	3	4	7	6	2	35
Other vegetables and fruits	8	4	2	3	6	6	3	5	13
Milk, cheese, ice cream	17	14	23	65	7	15	8	43	6
Eggs	7	3	8	3	10	7	6	13	0
Meat, poultry, fish	23	10	25	2	21	9	31	15	1
Butter and other fats	10	19	2	[b]	2	11	4	1	0
Sugars, sirups, sweets	3	8	[b]	2	3	[b]	[b]	[b]	1
Miscellaneous	2	1	1	[b]	[b]	[b]	[b]	[b]	[b]
Food adjuncts	4	No attempt to estimate nutrients in these							

[a] 35 per cent of flour enriched.
[b] Less than 0.5 per cent.

Cereal Grains and Their Products

For the majority of people in most parts of the world, the cereal grains and their products are the most abundant sources of food energy (calories) and of protein. When not too highly milled, or if "enriched" or "restored" according to present (U.S.A.) custom, this same food group is also a major dietary source of iron and of thiamine. Thus a fully appreciative use of this food group goes far to provide for four of the eight nutrient factors of the "yardstick" we here use, as may be seen in Table 59. And from Table 59 it will likewise be seen that this food group at a cost of 11 per cent of the total food expenditure furnished not only two to three times this proportion of the energy, protein, iron, and thiamine, but also nearly its full quota of riboflavin; and more than its full quota of calcium,

chiefly because of the use of milk in bakery products. The calciu and riboflavin of the grain products come in part from the grai themselves but also there is added calcium from milk solids a molasses in some bakery products and from the salt mixtures us to stimulate and regulate the action of yeast in breadmaking; a there is added riboflavin in enriched flour and bread, as well as fro the milk used in many bakery products.

Because the grain products bring the consumer so much nutrime for what they cost, they tend to be prominent in the dietaries of t poor. But whether the use of a high proportion of grain products a dietary or food supply is properly to be considered a cause of lo nutritional quality will depend upon how the grain products ha been processed, and upon how well this food group is balanced giving wise degrees of relative prominence to other food groups.

It is now entirely practicable to combine excellence of nutriti with improved food management by making fuller use of gra products than has been advocated hitherto when the prevalent form were nutritionally impoverished products.

Rice probably plays a larger part in the nourishment of the huma species, but the present discussion will center upon wheat becau it is our best-studied cereal and the dominant grain of those parts the world with which we have fullest acquaintance and in which th book will probably be mainly used.

All the major cereal grains and mill products have, in their usu air-dry state, very similar energy values at about 1600 to 18 Calories a pound, while bread because of its higher moisture conte has an energy value of about 1200 Calories a pound.

Protein values are fairly similar in the different *whole* cere grains. In each grain, however, there is present a mixture of protei some of which are of higher nutritive value than others. In genera the nutritionally better proteins are concentrated in the outer laye of the seed and in and around its embryo or germ. Hence the ord nary milling processes reject much of these more valuable protein and the resulting refined product becomes a nutritionally impove ished food,—even though in the case of wheat it retains the bulk the particular protein which confers the texture desired in brea

making. "Enrichment" of flour and bread, and "restoration" of breakfast foods, as officially defined, do not restore the protein values. Hence the often-used phrase "whole grain or enriched (or restored)" does not mean that the enriched or restored products are equivalent to whole-grain products in protein value. The protein value of white bread is, however, improved if milk solids or extra wheat germ are introduced in substantial proportion in the breadmaking.

Supplementary relationships. Osborne and Mendel found in experiments with rats that when white flour (wheat endosperm) was the sole source of protein there was practically no growth, while under parallel conditions the proteins of whole wheat supported good growth. Evidently the proteins of other parts of the wheat kernel (chiefly, no doubt, those of the embryo and of the bran) supplement those of the endosperm. The proteins of white flour are also effectively supplemented by those of meat, eggs, milk, soybeans, and peanuts. Moderate levels of protein intake support excellent growth when one third of the protein is from milk and two thirds from white flour or bread, and support nitrogen equilibrium when one tenth of the protein is from milk and nine tenths from bread, corn meal, or oatmeal. Repeatedly a mixture of one sixth dried whole milk and five sixths ground whole wheat has supported normal growth, health, reproduction, and lactation, through more than 70 successive generations of rats with no diminution of size or vigor though the protein level was only 14 per cent of the total food calories, and more than two thirds of this was wheat protein.

From the viewpoint of their agricultural and nutritional chemistry, we might well make a considerably larger use of the grain crops as human food than we now do.

In round numbers, the United States produces yearly about three thousand million bushels of corn (maize), about one thousand million bushels each of oats and wheat, about four hundred million bushels of barley, and about sixty million bushels each of rice and rye.

If after taking out seed for the next crop and the amounts of grains wanted for draft animals, the remainder were moderately milled for human food, these six major grain crops would together yield the

entire calorie need of 400,000,000 people or nearly three times ou population. Of course, grain products alone do not make an ade quate diet, but they could be adequately supplemented by the othe foods which our farms and ranges could still produce.

For, at present, an almost incredibly large proportion of grai crops suitable for human food, which our farms are producing, i being fed to meat animals. And this "processing" of grain crop through animals is inherently expensive of resources. The Food an Agriculture Organization of the United Nations estimates that seve "original calories" are eaten by farm animals for every calorie o human food produced through animal feeding. In this connection i should also be remembered (even at some cost of repetition) tha the milk animals make a much more favorable return than do th animals which are fed for slaughter.

A common but misleading ambiguity is the use of the term "feed grains as if these were significant only for animal feed, whereas i fact much the greater part of the grain fed to animals (that is, muc the greater part of the oats and corn) is quite suitable for huma food.

We can greatly improve the economy with which we use our grai crops by realizing that not only wheat and rice but also oats an corn are good food for man; and by growing and using more whea and oats for human food while subjecting a smaller proportion o our grain crop to the inevitably expensive "processing" of grain through animals. Wheat particularly may well take a larger place i our food economy, for its production can easily be greatly expande in the United States.

Potatoes and Sweetpotatoes

Though not related botanically,* potatoes and sweetpotatoes ar bracketed as a food group because both are rich in carbohydrates both high in energy value and capable of forming a large part of th diet of those accustomed to eat them, and because they are reason

* It is for this reason that sweetpotato is made a single word to mark it a an arbitrary and artificial term.

ably interchangeable in meal planning. Considering the quantities in which they may readily be eaten, both potatoes and sweetpotatoes can be important dietary sources of vitamin C when cooked and handled with reasonable regard for the conservation of this factor. They differ in that the vitamin A value is low in potatoes and high in sweetpotatoes. Potato protein has been shown to be of high nutritive efficiency; the proteins of sweetpotatoes do not seem yet to have been much studied in this respect.

From Table 59 it will be seen that, compared with its cost, the contribution of this food group to the nutritive value of the diet is large and varied. Only in calcium does it fall short of its quota. It furnishes somewhat over its quota of calories, protein, and riboflavin; and much over its quota of iron, vitamin A value, thiamine, and vitamin C. Americans might well make larger use of this food group. Our farmers could readily raise more. Our per capita consumption of potatoes is considerably below that of Great Britain and of Northern and Western Europe. And British experience during the Second World War shows that potatoes may safely be given a larger place in our food supply.

Mature Legumes and Nuts

Compared in the mature dry state, the legumes are about twice as rich in protein as are the cereal grains. The soybean is outstanding both for its high protein content and for the nutritive efficiency of this protein which ranks with those of meats, eggs, and milk, whether fed alone or as a supplement to the proteins of white flour or bread And peanut protein is almost as efficient as soybean protein in both these respects. The proteins of peas are again almost as efficient in nutrition. Regarding the nutritive efficiency of the proteins of ordinary beans, the literature has been confusing but the evidence is now becoming more clear. In some early experiments, beans fed raw as sole source of protein gave poor results; but when cooked and fed with any food which supplies some extra cystine or methionine, the protein of the common bean becomes nutritionally efficient. Universally beans are cooked before human consumption, and the

amino acid supplement which gives them good nutritional efficiency —cystine or methionine or usually a mixture of the two,—is widely distributed among our everyday foods. Even white bread thus supplements our common peas and beans,—though whole wheat or "Boston brown" bread does it somewhat more efficiently.

The customary combination of baked beans and brown bread makes a "main dish" that ranks with meat as a source of nutritionally good proteins and vitamins of the B group. If the brown bread has been made with milk and molasses, then it with the beans will constitute an excellent nutritional supply of the mineral elements also. The chief nutritional low points of such a one-dish combination are in the vitamin A and C values, the outstanding "primary sources * of which are the green and yellow vegetables, and citrus fruits, tomatoes, and "salad greens."

Seeds, roots, and tubers, while together furnishing 38 per cent of the calories, furnished also (in the study here discussed, Table 59) 36 per cent of the protein, 39 per cent of the iron, and 36 per cent of the thiamine; but furnished only about 17 per cent of the calcium, about 6 per cent of the vitamin A value, 16 per cent of the riboflavin and 13 per cent of the vitamin C. When, now, the green and yellow vegetables are included, we find that 39 per cent of the calories, 38 per cent of the protein, 22 per cent of the calcium, 45 per cent of the vitamin A value, 42 per cent of thiamine, 21 per cent of the riboflavin, and 44 per cent of the vitamin C have been furnished. Thus the addition of an average amount of green and yellow vegetables to the seeds, roots, and tubers brought everything except calcium and riboflavin to levels well balancing the calories.

The milk (Table 59) brings the calcium and riboflavin of the diet or food supply into a well balanced proportion and also provides a generous margin of animal protein in an exceptionally good form.

Meat, fish, eggs, fats, and sweets all thus turn out to be "marginal"

* Agricultural-economic terminology treats as "primary" the foods that grow on or in the soil, while those obtained through animals are "secondary" or "secondarily derived."

foods which we could share with others to any desired extent without danger to the nutritional adequacy of our American dietary. This the student may verify for himself by working out in more detail what we have here presented in somewhat sketchy fashion, the student being presumably now mature enough to find his own way forward in this analysis of the place of each food group in the dietary.

Green and Yellow Vegetables

The green and yellow vegetables are grouped together, for many practical purposes of dietetics and of crop production planning, because of their relative richness in vitamin A value; though they differ rather widely among themselves in this respect. There are also two noteworthy exceptions. Yellow turnips (including yellow rutabagas) owe their color to substances other than carotene and do not have the vitamin A value that their yellowness suggests; while sweet-potatoes that *do* have relatively high vitamin A value (varying with their intensity of yellow or orange color) are commonly bracketed with potatoes because of their high carbohydrate content and energy value and the food habits that make these alternatives with each other rather than with other vegetables in the planning of meals or of national or regional food supplies.

Some of the green vegetables are very noteworthy sources of vitamin C as well as of vitamin A. Even when they have been cooked (if intelligently and in moderation) such green vegetables as kale and turnip tops are still rich in ascorbic acid.

Recent years have seen a gratifying growth in appreciation of green vegetables. The increased production and consumption of green-stuffs is clearly indicated both by nation-wide marketing statistics and by the increased attention to home gardens.

As calcium is probably as critical a factor as vitamin A in American dietaries, it is certainly worthwhile to teach, clearly and patiently, that the green leaves whose calcium is available, such as broccoli, collards, kale, loose-leaf lettuce, and turnip tops, are importantly preferable to spinach and other members of the Goose-

foot family, because these latter contain so much oxalic acid as to render their calcium of little if any use. With the green leaf foods, it is especially true that differences *within* as well as *between* food groups may be important.

Citrus Fruits, Tomatoes ("Salad Greens")

Citrus fruits and tomatoes well deserve special recognition as sources of vitamin C, for their content of this vitamin is relatively high and they hold it well in storage or cooking or canning.

Tomatoes can be grown on almost any kind of soil and throughout a wide range of climate; and successfully by beginners. They are "one of the main standbys" of home gardens. Also, the tomato is a good source of vitamin A as well as of vitamin C, and a fair source of riboflavin. The simple step of using, for the raising of extra tomatoes, an extremely small fraction of the land hitherto devoted to cotton, would doubtless mean an important improvement in the Southern food supply.

Citrus fruits have already become about as cheap as most other fruits in many of our large cities, and the greatly expanded areas of citrus orchards in Florida, California, and Texas give good assurance that from now on oranges and grapefruit are to be regarded as staples, not luxuries. As may be seen from tables in Chapter 17 and Appendix, broccoli, cauliflower, kale, and turnip tops are, when fresh and raw, sufficiently richer in this vitamin than are oranges so that even after moderate storage and cooking they may still be regarded as comparable sources. It is also worthwhile to consider a larger use of these vegetables raw as salad greens and relishes.

Other Fruits and Vegetables

The fruits and vegetables other than those belonging to any of the four preceding groups include a large number of items most of which are too seasonal to be large contributors to the nutritive value of the year-round diet, though apples and bananas may now be regarded as almost constantly available in many American mar-

kets. Naturally, the segregation of the fruits and vegetables of outstanding nutritive values, in the groups previously given, leaves a number of rather expensive specialties in the residual group. It is, however, a good source of vitamin C and a fair source of other vitamins and of the nutritionally important mineral elements; and is also of value in giving wholesome variety to the diet.

Milk and Its Products Other than Butter

Milk is the one article of diet whose sole function in nature is to serve as food. In addition to being an excellent source of energy, protein, and of mineral elements and vitamins generally, it is the outstanding source in American dietaries of calcium and riboflavin, the two nutrients in which our dietaries most often need enrichment according to the Recommended Allowances of the National Research Council. These facts suggest strongly that this food group might well be given a higher place than at present in American dietaries.

Comparing the money cost of the milk group in the diet shown in Table 59 and its return in nutrients, we find as follows: This food group represented an investment of 17 per cent of the food money, in return for which it furnished 14 per cent of the calories, 23 per cent of the protein, 65 per cent of the calcium, 7 per cent of the iron, 15 per cent of the vitamin A value, 6 per cent of the vitamin C, 8 per cent of the thiamine, and 43 per cent of the riboflavin. Hence the nutrient return is well over the cost; and in addition this food furnishes the nutrients in more favorable forms than does the dietary as a whole, and strengthens it at the points where such strengthening is most important to the people of the United States as consumers.

Milk sugar is an advantageous form of fuel food in being non-irritating to the stomach, and requiring only a single simple hydrolysis for its digestion.

Milk fat contains nutritionally essential fatty acid and is more readily digested than the fat of most foods because it is in an already emulsified form.

The protein of milk is easily digested, is less liable than other animal proteins to putrefactive decomposition in the digestive tract, and ranks among the most efficient of proteins in nutrition both by itself and as supplement to the proteins of bread and other grain products.

The richness of milk in calcium and riboflavin is important for the reason already suggested and may also be considered a "hall-mark" of excellence in well balanced mineral and vitamin content. Still valid is the concise summary of the late Dr. Mendel of Yale: That milk contains all the known vitamins; and that, while each of the vitamins may be found in higher concentration in some other food, it is doubtful if any other food furnishes so many of these essentials in so nearly well-balanced proportions as does milk.

Eggs

In a general way, and in some particular ways, nutritionally, eggs stand in a position intermediate between meat and milk. In some other ways, eggs are more or less unique in their properties as food. They are highly prized by a large proportion of people, and the laying hen transmits as human food in the form of her eggs a larger proportion of the nutrients she consumes than do most meat animals. Quantitative comparison on this point is difficult because of differing estimates of the normal average productivity of the hen, and also because in the nature of the food which she requires in order to do her best, she competes more directly with human food requirements than do most classes of livestock.

The net outcome as regards the economy or costliness of eggs compared with other foods may be judged by noting their percentage share of the total cost of food and the percentage of each food factor or nutrient that they furnish, as illustrated by the data of Table 59 above.

Besides the relative costliness of eggs, there are other considerations to be weighed in assessing their nutritional characteristics and desirable place in the diet. They are rich in proteins of similar high nutritional efficiency to those of meat and of milk; but egg protein

more often undergoes putrefaction in the intestine than does milk protein. Eggs are rich in iron and phosphorus, but their mineral content as a whole is not well balanced. While rich in some vitamins they are practically devoid of some others; and they are rich in cholesterol which, while it functions in our bodies, may be received in larger than optimal amounts. There is need of research upon the long-run effects of different levels of egg consumption. Such research should clarify the question whether in such grouping of foods as that of this chapter, eggs should be made a group by themselves or included with meats, fish, and poultry.

Meats, Fish, and Poultry

American food budgets show on the average that about one fourth of the expenditure for food is for meats, including poultry, fish, and shellfish. All such statements must be construed flexibly because such fat meats as bacon and salt side are sometimes included among meats and sometimes among fats. In Table 59, the "meat group" does not include these fat meats: With this in mind, note the percentage of the total food money that this group costs, and the percentages of the total of each nutrient that this group furnishes.

In general statements about the nutritional characteristics of meats as a group compared with other food groups, it should be kept in mind that usually 97 to 98 per cent of the total market meat is muscle meat and 2 to 3 per cent is liver (perhaps including some other "variety meats"). Hence the contribution of the meat group is very predominantly that of the muscle meats.

The conventional grading which puts a premium upon grain-fed beef is both extravagant of resources and out-of-date from the nutritional point of view, forage-fed beef being decidedly superior to grain-fed beef in vitamin A value. Cabell, Ellis, and Madsen (1943) show that this is true of both the fat and lean portions, and that the edible fat of beef cuts may be twenty times as rich in vitamin A in pasture-fed as in heavily grain-fed cattle.

Meats are rich either in proteins or fat, or in both. Meat proteins

are of high nutritive efficiency either separately or as supplements to bread protein.

Along with their proteins, lean meats contain considerable amounts of riboflavin which seems to be present as a fairly constant proportion of the lean muscle tissue, though somewhat influenced by the food of the animal. Thiamine is also present in the muscle tissue but in quite different proportions in different species, pork containing much more than beef in muscles of similar fatness. In the meat animals generally, liver is richer than muscle, weight for weight, in both thiamine and riboflavin; but the proportion of liver to muscle meat is too small for the composition of the liver to have much effect upon the nutritional character of the meat supply as a whole. The distribution of other vitamins in meats has been discussed to some extent in previous chapters and is treated in detail in some of the suggested readings.

Both in their vitamins and in their mineral elements, there is a strong general resemblance between the meats and the cereal grains. Both are relatively rich in the B vitamins (though the lean meats are richer in riboflavin than the grains), while relatively poor in vitamin A value and vitamin C content. Both contain fair amounts of iron and phosphorus but are poor in calcium and the base-forming elements generally.

Thus it is chiefly with reference to the factors concerned in the older knowledge and the prescientific traditions of food and nutrition that the breadstuffs and meats seem to supplement each other. The former are richer in carbohydrate, the latter in fat or protein or both; so that meats (differing from bread and potato in being rich in fat or protein or both and also in the possession of characteristic flavors, well liked by most people) have a special sort of "satiety value." Thus to many people a liberal amount of meat, rich in both protein and fat, increases both the "satisfying" and the "staying" effect of a meal. How far this effect is really nutritional, and how far it is more scientifically attributable to a combination of sensations, is still an open question. And any scientifically serious study of this question will reveal that it is further complicated by the long-standing tradition that treats meat as the "main dish" of a

dinner and associates a high meat consumption with a high standard of living. Furthermore, if one could disentangle the complications of tradition and psychological reactions from the nutritional effects of the level of meat consumption, there would still remain the very important problem, how to give well-balanced consideration to the short-time *versus* the long-time nutritional effects. In view of the fact that our national meat bill takes a high percentage of our income, it may be surprising how little has yet been done in the use of comprehensive and long term methods of nutritional research to throw light on the question, What difference would it make in our life histories whether our level of consumption of meat were habitually higher or lower throughout the life-time?

Food Fats

Fats are widely distributed in the organs of both plants and animals, and so are contained in varying proportions in nearly all the natural foods that we eat. The fat which thus comes into consumption as an unseparated part of a natural food is called "invisible fat" in contradistinction to the commercial fats that we buy as such. We have already seen that it is not always practicable to draw a sharp line between fats and fat meats. Clear fat cuts of pork, and even predominantly-fat bacon will sometimes be found classified as meats and sometimes as fat. There is no distinction between fats and fatty oils as food. The commercial distinction shows only whether the fat is solid or fluid as ordinarily handled.

The outstanding nutritional characteristic of the fats is, of course, that they constitute our most concentrated food-fuels. In this fuel function, all the fats we use as food are essentially alike. The properties of fats and the fate of fat in the body have been described in Chapters 3, 6, and 7 and need not be reviewed again here.

Because of the difference in chemical nature and nutritive function between lean meats and commercial fats, some nutritionists felt misgivings when these two types of food were, as a war measure, submitted jointly to the same "point rationing." But it turned out that even though some families used more of their ration points for

meats and others for fats, neither was in any danger, because our (U. S. A.) levels of consumption were still nutritionally liberal in both meats and fats.

In vitamin A value, commercial fats range from practically none in most shortening fats and salad oils to an average of about 15,000 to 16,000 I.U.* per pound in butter. The present (1951–52) standard for fortified margarine is 9000 I.U. per pound; but most margarine on the American market is said to contain 15,000 I.U. per pound.

Sugars

As fats are our most concentrated, so sugars are our "quickest," fuel-foods. Sugars as food have been as fully described in Chapters 2, 6, and 7 as space in this small book permits.

Explanation

Space is not available here to discuss the food groups in technical detail, yet there seems need for the foregoing brief sketch to facilitate the transition of our viewpoint from the more chemical study of foods in terms of their constituents to the more largely economic consideration of foods as agricultural products and as trade commodities, and to the study of the causes and extent of variations. There follow suggestions for readings which will amplify the student's descriptive acquaintance with foods in almost any direction desired.

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* I.U. is the accepted abbreviation for International Unit, which as explained in Chapter 23 is of the same value as the U. S. Pharmacopeia (U.S.P.) Unit.

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Chapter 31

CAUSES AND EXTENT OF VARIATIONS IN THE NUTRITIVE VALUES OF FOODS

Variety

In different specimens of food of the same kind, variations in composition and nutritive value may be either varietal (genetic) or may be due to environment. Sometimes the one and sometimes the other cause of variation predominates. The immediately following studies illustrate varietal differences:

Batchelder (1934), in a compilation of data from other laboratories as well as her own, found 14 varieties of apples averaging respectively 16.7, 12.5, 11.1, 5.0, 5.0, 5.0, 5.0, 4.5, 3.9, 3.7, 2.7, 2.5, 2.5, and 2.2 milligrams of vitamin C per 100 grams of the edible portion. (Note the more frequent occurrence of values near the middle of the range.) Thus different varieties, which were originally developed for the sake of other qualities, now turn out (in some cases, but not all) to differ widely with respect to their richness in vitamin C. This is also true, though usually in a lesser degree, of the same and other vitamins in different varieties of other species of food.

Fincke (1939) found in peas that 10 varieties averaged, respectively, 740, 710, 670, 650, 520, 500, 430, 360, 330, and 200 micrograms of thiamine per 100 grams of the fresh green immature peas.

Murphy (1941) found that 16 varieties of fresh, mature onions showed under comparable conditions, 40, 36, 34, 33, 31, 30, 30, 27, 22, 21, 21, 20, 20, 19, 19, and 17 milligrams of vitamin C per 100 grams, respectively.

Schultz, Atkins, and Frey (1941) found in 28 varieties of wheat, 730, 710, 690, 670, 670, 630, 610, 610, 610, 600, 600, 590, 590, 590, 580, 570, 520, 520, 510, 500, 470, 470, 450, 450, 450, 450, 450, and 420 micrograms of thiamine, respectively, per 100 grams of the air-dry grain.

In all the above cases the average for each variety is given sepa-

rately in order to show not only the range but also the distribution within the range,—the extent to which specimens are naturally bunched around some median point.

Influence of Earliness, Maturity, Size, Exposure to Sunlight, and Storage *

In the work of Tressler, Mack, and Jenkins (1937) with lima beans, and that of Todhunter and Sparling (1938) with peas, higher percentages of ascorbic acid were found in the smaller than in the larger vegetables of the same variety and maturity. But with tomatoes no similar constancy of relation between size and vitamin C value was found by Tripp and Satterfield (1937), although in another series of observations the concept of an inverse relationship between size of fruit and concentration of the vitamin appeared to be supported (Brown and Moser, 1941). Probably this is because, as later work has emphasized, the vitamin C content of tomatoes is so greatly influenced by weather conditions, especially direct sunshine, that this may have offset the weaker tendency toward an inverse relation to size.

Mack, Tapley, and King (1939) found that among ten varieties of snap beans the earlier varieties tended to have higher vitamin C values at the given stage of maturity.

Mack, Tressler, and King (1936) found among peas that in general the early, small-seeded varieties are richest in vitamin C; but also that the smaller peas had higher concentrations of this vitamin than the larger peas of the same variety. In all the varieties studied, the concentration of vitamin C in the seed decreased with advancing maturity. Storage for six days at 1° to 9° C. resulted in no significant decrease of vitamin C value whereas parallel lots of the same fresh peas showed "very considerable losses" when kept the same length of time at 18° to 22° C.

Tressler, Mack, and King (1936) studied the influence of several factors upon the vitamin C content of spinach leaves. Studies on

* Where studies of storage have been incidental they are included here instead of repeating the description of the research in another section.

two varieties throughout the cutting season showed no difference attributable to stage of maturity within the range through which spinach is eaten. Losses of vitamin C value in storage were greatly influenced by the temperature: The spinach stored at 1° to 3° C. lost only about 4 per cent in 3 days, while at 23° to 26° C. (room temperature) about 50 per cent was lost in the same 3 days. Nearly all the vitamin C of spinach disappeared during 7 days storage at room temperature (93 to 96 per cent at 23° to 26° C.). Contrast this with the relatively high stability of the vitamin C values of tomatoes and citrus fruits.

As Adams and Smith (1944, pages 45–47) have well emphasized, vitamin losses in storage may be small or large depending on storage time and conditions, the commodity concerned, and the vitamin in question. Usually we may expect larger loss of vitamin C than of any other, and this is the vitamin on which most studies have been made. The rate of deterioration of vitamin C in food has been found to be influenced by many factors. In the great majority of cases, the other vitamin values are probably better conserved.

Smith (M. C.) and Caldwell (1944)* studied the ascorbic acid content of oranges from four navel orange trees grown in the experimental orchard at Yuma, Arizona, and picked at weekly or biweekly intervals from the north, south, east, west, and center of the trees over a four months period of marketable maturity (October to January). The fruits from the shaded center of the trees were larger and yielded more juice at an earlier maturity than the other fruit on the same trees; but the smaller fruit, more exposed to the sun, contained a higher concentration of ascorbic acid in the juice. Both with the navel and Valencia oranges, it appeared that fruits from the center of the tree were larger and their concentration of ascorbic acid lower. The effects of such kinds and degrees of environmental control were of the order of twenty to forty per cent of the vitamin C content of these Arizona oranges.

Maynard, Beeson, Hamner, and coworkers found that the vita-

* Mimeographed Report #68 of the University of Arizona Agricultural Experiment Station, *Experiment Station Record* 92, 868–869.

min C content of tomatoes was more influenced by sunshine than by any other of the many other treatments they tried.

In studies of the influence of maturity in cantaloupes, Smith, Burlinson, and Griffiths (1943) found that one variety, "Arizona strain No. 45," increased in ascorbic acid content from an average of practically 30 milligrams per 100 grams of edible portion, in green fruit almost full grown, to an average of practically 40 when fully ripened. It fell rapidly when the fruit was distinctly over-ripe. At their best stage of maturity for eating, these cantaloupes ranged from 40 to 60 milligrams of vitamin C per 100 grams of the edible portion.

Soil and Fertilization

Swanson, Stevenson, Haber, and Nelson (1940) found no significant effect upon vitamin A value of sweetpotatoes from fertilization of the soil in which they were grown, with nitrate, phosphate, potassium salts, or stable manure. Reder, Ascham, and Eheart (1943) in a very extended and systematic cooperative study of the influence of fertilization upon the vitamin C (and calcium and phosphorus) contents of turnip greens showed variations apparently attributable to many different circumstances, but no such conclusive differences or clear-cut trends as would warrant emphasis as definite guides.

Hamner, Lyon, and Hamner (1942) found surprisingly little relation between any of many fertilizer treatments (salts added to the soils, sand culture, or nutrient solutions in which the plants grew) and the ascorbic acid contents of tomatoes. Location where grown seemed to have some effect but this did not appear to depend upon the soil. Plants grown in sand culture supplied with a balanced nutrient solution produced tomatoes quite as rich in vitamin C as those grown on good soils. Their paper gives a full account of the many experiments they tried with different salt fertilizations.

Maynard and Beeson (1943) summarize the evidence on the subject by saying that several investigators have sought but have "found little or no effect of fertilization on the ascorbic acid content," of a

number of vegetables. They also express the opinion that if soil type exerts any effect upon the vitamin content of the crop, this could only be established by experiments in which many other factors are controlled. And they consider that in general the effects of soil type and fertilizer are less than those of variety and climate. They add: "It is clear, however, that only a very few of the many possible relationships have been studied."

Dependability of Average Figures for Mineral Elements and Vitamin Values in Foods

The *Plant, Soil, and Nutrition Research Laboratory* established and conducted at Ithaca, New York, by cooperation of the United States Department of Agriculture, the Agricultural Experiment Stations of the North-eastern States, and Cornell University, specializes in the study of variations and interrelationships with the practical objective of developing foods of improved nutritive values.

The problems involved are proving to be even more intricate than had been anticipated, and will doubtless require many years of research. Meanwhile the majority of our people derive the mineral elements and vitamin values of their dietaries chiefly by purchase in markets which draw their supplies from widespread areas where food crops have been produced under differing environmental conditions and by culture of differing varieties of plants and animals.

Thus there are (and doubtless long will be) many and important occasions for the use of average mineral and vitamin values of foods as consumers obtain these in markets supplied by different varieties and from different environmental conditions of production and marketing.

Studies of processing-losses should be more fully controllable than those of natural variations can be, yet even here the causes are so often complex and interacting, that many an investigation, carefully planned and conscientiously executed, yields findings that should be only tentatively interpreted because of internal indications that the outcome has been influenced by some unknown factor or factors. Obviously, in such cases the work should be repeated

with still stricter control of conditions, and perhaps also larger numbers of samples or observations, before a definite conclusion is drawn.

Environmental vs. Varietal Differences

When one starts to differentiate between kinds of the same species, it seems natural and logical to classify first according to named varieties. For this, being a genetic classification, seems more fundamental than divisions according to environmental factors. Actually observed data, however, indicate that we must be prepared to find that sometimes a genetic and sometimes an environmental factor predominates in determining natural variations of chemical composition and nutritive value.

Todhunter (1942) found with rhubarb, as did the workers in Maynard's laboratory with tomatoes, that the field-grown crops were consistently richer in vitamin C than those of the same varieties grown in hothouses. Often, indeed, the difference thus due to outdoor summer, as against hothouse winter, atmospheric environment of growth, was sufficient to overshadow varietal differences, which in turn may (sometimes, but need not always) exceed those due to fertilizer and soil type.

Importance of Numbers of Cases

When Dr. Stiebeling reports that about a thousand cases have contributed to the establishment of the vitamin C content of raw potatoes at a year-round average of 17 mg. per 100 grams of the edible portion, no one calls for more cases on this particular point. Also when one finds the calcium and phosphorus contents of cows' milk so regularly given as 0.118 and 0.093 per cent respectively, as they are, the present arrays of about 300 cases each may be considered as accepted for averages, at least for North America and Western Europe. But until similarly accepted averages are reached, authors and editors should cooperate to see that published papers on composition of foods *include individual data* to facilitate more

comprehensive averages, as the volume of trustworthy data increases.

Variations between Tables of Composition of Foods

At many points there may be small differences between the data in the "text tables" in individual chapters of this book, those in the Appendix, and those in the *United States Department of Agriculture Handbook*. And this should continue to be so, because in every such table the averages will change as knowledge grows, and so will be revised from time to time, as successive editions are published. See also the discussion accompanying each table in the Appendix.

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Chapter 32

FOOD ECONOMICS IN THE LIGHT OF THE NEWER CHEMISTRY OF NUTRITION

Nutritional Research Helps to Reform "the Backward Art of Spending Money"

One school of economic thought criticizes another on the ground that it "knows the price but not the value." A distinguished economist has written an essay (so well regarded as to be republished at least a decade later) on "The Backward Art of Spending Money." Its argument is that the "exact sciences" of physics and chemistry guide efficiently and progressively the productive industries by which goods, and through which incomes, are made; while the spending of the income, in the hope of obtaining thereby a satisfying life, remains a backward art because it has the guidance of only such inexact sciences as psychology and sociology.

In the years since that essay was reprinted, chemistry has accomplished more than could have been foreseen to guide the use of that most outstanding of all material resources, the food supply, in such manner as to make healthier and happier lives, more satisfying and of greater social value.

The spending of money for food, and the judgment of the proper place of food in the total budget of the individual or the family have both been greatly enlightened by recent chemical investigations of nutritive values and of the relations of nutrition to health and to the extension of the prime of life.

As our Government has officially recognized, economic conditions and the growth of scientific knowledge have focused attention upon the problems of food budgets. Consumers, interested in getting good returns for what they can afford to spend, wish to have the information interpreted at several economic levels; and producers

want to know how much of different foods may well appear in the diets of different consumer groups and to what extent consumption may be expected to rise or fall as the economic situation changes.

While our newest nutritional knowledge affords fuller and clearer guidance for the problem of the best investment of money in food, it can hardly be said that the problem has been made easier. The opportunity is stimulating but the responsibility is heavy. Under the best economic conditions which we can foresee for the near future, it will still be a real responsibility for every home-maker to feed her family in such a way as to give fullest nutritional support to the innate potentialities of each person. Yet we can approach much more closely to that goal than we now do. Here there is a three-fold need and opportunity.

(1) Openminded and thoroughgoing study is needed, of the individual or family problem as to how the money which is *being* spent for food may now best be invested. There is need to emphasize the fact that this is a question to which fundamentally important new evidence has been brought by very recent research. So far as space permits, the new evidence has been mentioned in previous chapters, and here we are considering how this newer knowledge should function in our scientific attitude toward the everyday choice and use of food, and what may be expected of a more scientifically guided food habit. Certainly a large majority of individuals and families may expect, in the course of a life-time, important benefits from the forming of such daily food habits as make use of this newest knowledge of nutrition.

(2) Because the potentialities of nutrition for the improvement of the life process and for the individual and family satisfaction in life are now found to be greater than expected, there is need for reappraisal of the *relation between expenditures for food and for other things*. The rather prevalent attitude of dismissing this problem on the ground that "the American standard of living demands" such-and-such expenditures in other directions, becomes no longer valid when one grasps the fact that a moderate diversion from expenditure in some other direction for the sake of increased investment in food under the guidance of the newer knowledge of nutri-

tion has a very high potentiality for bringing increased satisfaction in life.

(3) The same nutritional improvement of the life process, which may mean so much to oneself, also increases the economic and social value of the individual to the community and so builds a stronger nation of abler, happier, and more useful citizens. Hence the public health and general welfare of the nation demand that we extend to all people the newer and higher standards of nutritional well-being. True as it is that the dissemination of modern knowledge of nutrition and food values, even in very simplified form, will enable most home-makers to improve the feeding of their families on their present incomes; yet it is also true that some families have incomes so small that they could hardly be expected to be so fed as *fully* to develop their innate potentialities, however well instructed and conscientious they are in the choice and use of food. If the nation is to protect its own economic and health interests, there must be effective recognition of the fact that *the problem is both educational and economic*. Better levels of consumption of the right kinds of food should be taught to all the people; and, in addition, *should be made possible* for those of low income either by increasing their purchasing power or by subsidized distribution of the actual articles of food whose increased consumption is most directly indicated by nutritional knowledge. Improved nutritional status is itself an important factor in the increase of earning power.

That the newer knowledge of nutrition is influencing the people of the United States in the direction of better use of food is clearly shown by the food statistics of the agricultural economists and by surveys of family food consumption. But the evidence is also clear that further improvement is needed.

From data given by Phipard of the Bureau of Human Nutrition and Home Economics, there are reproduced in Table 60 the levels of consumption found in 1936 and 1942 of the three groups or types of food which we would most emphasize in the light of the recent growth of nutritional knowledge.

These are gratifying increases in the use of all three of the types of food whose increased consumption is nutritionally most to be

TABLE 60. U. S. FARM FAMILIES: PER CAPITA CONSUMPTION OF CERTAIN FOODS

TYPE OF FOOD	1936	1942
Green and yellow vegetables, *pounds*	57	95
Citrus fruits and tomatoes, *pounds*	43	84
Milk,[a] *quarts*	226	294

[a] Including canned and dried milks, cheese, and cream as milk equivalents.

desired. Enough less of some other foods was consumed so that the total calorie intake was essentially the same; but the better food selection in 1942 than in 1936 resulted in substantially better intakes of calcium, vitamin A value, thiamine (partly due to enrichment of flour and bread), riboflavin, and ascorbic acid. Also the 1942 dietaries were slightly the richer in protein, iron, and niacin.

With increased income, a part of the increase is used to buy more of the food which the people concerned most enjoy eating; but at any given level of income the accustomed expenditures for other things are too apt to be regarded as fixed by "the standard of living." And not only in the food trades but also among those who teach or investigate food science or food economics there often appears a sort of "vested interest" attitude of resentment against any teaching of readjustment of consumer's expenditure.

For such reasons it may be worthwhile to emphasize again the fact that we can use the guidance of the newer knowledge of nutrition and continue to eat every kind of food. As McCollum teaches: Eat what one should, and one may also eat what one likes; or, "Eat what you like so long as you eat what you should."

As we have seen, the development and diffusion of nutritional knowledge is already affecting the relative prominence of some articles of food in the typical consumer's budget, and such adaptation of emphases in food habit to our growing nutritional knowledge will probably go farther; while every kind of food we now know will doubtless continue to be produced and distributed and so to be as readily available to consumers as it now is. Simply, relative amounts consumed can and should be shifted somewhat in the light of today's knowledge of nutrition and of its importance to health and well-being.

When nutritional science indicates that a certain type of food should have a more prominent place in our food economy than it now has, this is guidance offered both to producers and consumers. Nutritional guidance should appeal first of all to the consumer. Then, increased consumer demand means a "better market" for the producer and so encourages him to increase his production of that food.

The classification of food commodities into the *ten or eleven food groups* discussed in Chapter 30 is a great help in food economics. If the particular kind of food that the consumer had in mind is not readily available in her market that day or if the price is higher than she feels she can afford, some other food of the same group should be a satisfactory alternative both nutritionally and from the viewpoint of meal planning.

All such plans aim to give sound advice in food economics guided by nutritional knowledge; but most of them aim also to be as considerate (or indulgent) as possible of current food habits,—to ask of their readers only an easy step in the direction that scientific knowledge of nutrition clearly indicates. The homemaker scientifically informed as to nutrition and food values may well ask herself whether she cannot take a somewhat more substantial step in the direction indicated by nutritional science, buying more fruit, vegetables, and milk, with less of something else, than most current plans suggest. This should result in more benefit to nutritional well-being at the same money cost and with no serious psychological difficulty when the gain to family well-being is understood.

Using data from the tables now readily available, students may easily compute what they obtain in nutritive values from their own expenditures for foods. This is the most important food question for the consumer. Too much of the advice currently addressed to consumers is written as if the consumer's problem were chiefly one of avoiding being deceived by the seller, whereas in fact a much more important consumer problem is that of deciding what things to buy and how much of each. Not only is the latter the more important, it is also the more permanent, consumer problem. For the food laws with their National, State, and local inspection services

are, together with Federal trade regulation, steadily relieving the consumer of the police function of preventing fraud; while decisions upon how to invest the food money remain a function of the consumer, and are steadily growing a more and more weighty responsibility as research reveals the unexpectedly great and far-reaching influence of nutrition upon health, efficiency, earning power, satisfaction in life, and social value to one's community and times. Thus the fact bears repetition that the best thoughts of the consumer should be devoted to making full use of scientific guidance in deciding what foods to buy and in what relative proportions to buy them.

When the newer chemistry of nutrition suggests a greater prominence of fruits, of vegetables, and of milk (including cheese, cream, and ice cream) in the American dietary, it is not suggesting anything untried. Gradually increasing numbers of American families have already found satisfaction in this more modern plan, and in the superior health and efficiency which it induces. A gradual trend of the national food consumption in the direction indicated as beneficial by our newer nutritional knowledge means that the example of those who are already benefiting by this knowledge is being followed by one after another of their neighbors.

And the trend to greater prominence of fruits, vegetables, and dairy products in the family dietary and national food economy is progress for agriculture also. As expressed in an official publication of the U. S. Department of Agriculture, the general use of such dietaries "would not only improve the health and efficiency of the population, but at the same time would foster the type of agriculture which represents wise utilization of land for the country as a whole."

Partly but not entirely because of the recent evidence that the optimal calcium intake is significantly higher than is provided by hitherto approved dietary standards, increasing numbers of people guided by this newer chemistry of nutrition will doubtless follow the advice frequently given by McCollum and include a quart of milk per day in their customary food consumption.

Together with increased milk consumption there will probably

continue to go an increased consumption of fruits and vegetables, especially the citrus fruits and the green vegetables: hitherto regarded as luxuries but now seen to be good dietary investments. Because fruits and vegetables differ so much among themselves and in the ways that they are served, it is harder here than in the case of milk to compare the consumption levels of different individuals or families in as accurate a way. Yet there need be no doubt that with growing acceptance of the guidance which our newer knowledge offers, fruits and vegetables are being given an ever greater prominence in the dietary.

Thus one student of nutrition consumed in the course of a recent year: 1577 servings of fruit and fruit products; 275 servings of tomato or tomato juice; 1407 servings of other vegetables. During the same year the total consumption of meats, poultry, fish, and shellfish, by the same individual was 300 servings; of eggs, 116 servings. The servings of fruits, vegetables, and their products were of at least average conventional sizes; so that probably the 3259 servings or portions consumed in the year represented at least 800 pounds a year of fruits and vegetables as they come to the kitchen, or the same as the highest levels of fruit and vegetable consumption that the writer has seen recommended by the U. S. Department of Agriculture. In this same dietary, the servings of meat averaged less than conventional size: probably meat consumption here was near the level of the lowest figures which the Department of Agriculture has yet recommended. Here a food habit guided by the newer knowledge of nutrition and giving more than average prominence to fruits, vegetables, and milk, at the same time afforded greater pleasure than the more conventional American food habit of the past, and at less cost. Later, the same student of nutrition found that the place of fruit in his dietary had progressed to a higher level. In a year in which they were especially abundant, he consumed 868 pounds of citrus fruits and their juices as well as usual amounts of other fruits.

Certainly many and probably most families and individuals could build health to higher levels by giving to fruit and to milk in its various forms a larger place than they now hold in the dietary. This

is possible, even with present purchasing power, by moderate readjustment of expenditure: partly by shifts within the food budget; partly by economizing in other directions in order to have more to spend for food. Cost-of-living studies show that even among people of the lower income groups some money could be shifted to the purchase of more fruit and of more of whatever form of milk, cheese, cream, or ice cream one likes best, from present expenditures for adornment-other-than-clothing and amusement-other-than-automobile. Once the significance of the gains to health and efficiency is understood, this does not mean deprivation but rather increase of satisfaction. Fruits and dairy products can be so selected and used as to add much to the pleasures of the table. And the satisfaction of improving the buoyancy of health, the efficiency, and the level of achievement of all its members is the highest and most lasting pleasure that a family can derive from its income.

Significance of Home Gardens

Doubtless it is true that what is grown in home gardens will have no appreciable effect upon any important food industry. Recognition of this should reassure the agricultural economist and prevent undue expectations in the home gardener. The agricultural economist need not fear, and the home gardener will be wiser not to hope, that the products of home gardens will materially influence the food markets.

Yet the home gardens can be of very real and significant benefit through the fact that each family with a garden can grow an *extra* supply of fruits and vegetables to be consumed *in addition* to what they have been accustomed to feel able to buy. This can mean very material improvement of nutritional well-being and resultant health, happiness, and efficiency; and the means for it can be found by a constantly increasing proportion of people as they come to realize how great a benefit it can be to themselves and (still more) to their children.

An important point here, which could not be clearly realized before we had the light of the newer chemistry of nutrition, is that,

when the demand of the market has been fully met, in the agricultural-economic sense that consumers have bought all that they feel able to buy at a price at which producers and distributors can afford to sell, the average consumer family still does not have as much of fruit and fresh vegetables as it needs if it is to realize its full potentiality of health, efficiency, and earning power.

Dietary Adjustments and the National Food Supply

As has been briefly noted earlier in this chapter, the general adoption of a dietary such as the newer knowledge of nutrition teaches would undoubtedly help the natural evolution of American agriculture. For a given amount of materials consumed, a dairy herd yields a product of much greater food value than does a herd of beef animals. Armsby estimated that of the energy value of what the animal consumes, about 18 per cent is recovered for human consumption in milk and less than 4 per cent in beef. According to estimates of the U. S. Department of Agriculture, the returns in human food protein are from three to five times higher through milk production than through meat production. In the conservation of calcium and of vitamin A values the difference in favor of the dairy animal is still greater. With the development of a more intensive agriculture, the inherently greater economy of dairy cattle over cattle raised merely for slaughter naturally tends toward a shifting in the proportions of the two types. Regions adapted to dairy farming but too remote from large markets to ship milk in the fresh state, can now market their dairy production as canned and dried milk; and also, of course, as butter and cheese. Yet because of its great areas of range pastures, America will continue to have an abundant meat supply as compared with any reasonable concept of requirement. And the country is learning to shift its emphasis from grain-fed to grass-fed beef—a shift which nutritionists hope will go farther.

Relative Economy of Different Food Groups

Using the data of the U. S. Department of Agriculture summarized in Table 59, we find that grain products at a cost of only

11 per cent of the total food money furnished 30 per cent of the calories, 28 per cent of the protein, 12 per cent of the calcium, 21 per cent of the iron, 22 per cent of the thiamine, and 9 per cent of the riboflavin of the diet. Even though the grain products furnished no significant share of the vitamin A or C value, they brought into this food supply a much larger total contribution of nutritive value than their share of the cost.

In data from the same source we find that with only 1 per cent of the food money spent for dry beans, peas, and nuts, these brought to the food supply 3 per cent of its calories, 5 per cent of its protein, 3 per cent of its calcium, 11 per cent of its iron, 6 per cent of its thiamine, and 3 per cent of its riboflavin. The potato-sweetpotato group, while not so outstanding, is still decidedly a bargain when all its contributions are considered.

Those who wish may readily work out which of the food groups are bargains and which are comparatively costly in such an over-all view of their costs and contributions.

Those who have studied this book thus far should find it easy and interesting to extend such studies to the economics of the individual food habits and the significance of differences both between and within the food groups.

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Chapter 33

TRENDS OF FOOD CONSUMPTION

In the summer of 1949, the United States Department of Agriculture published a more complete report than any previously made upon *Consumption of Food in the United States, 1909–48*. This with its *Supplement for 1949*, published in September 1950, constituted a fuller accounting for our national food supply than had previously been undertaken.

Tables 61 and 62 show respectively (1) the annual per capita consumption of major foods or groups, and (2) the daily per capita intake of the outstanding nutrients or nutritional factors. In each case the data for 1909 and for 1949 are shown side by side to illustrate the trends of food consumption in the United States during the intervening 40 years.

Some will welcome, and others will regret, the decided downward trend of consumption of grain products and of potatoes and

TABLE 61. TRENDS OF PER CAPITA CONSUMPTION OF FOOD BY MAJOR GROUPS: IN THE UNITED STATES

	1909	1949	1949/1909
	Lb.	*Lb.*	
Dairy products, excluding butter	388	429	111%
Eggs	35	46	131%
Meats, poultry, and fish	161	159	99%
Fats and oils, including fat cuts and butter	59	65	110%
Dry beans and peas, nuts, soya products	10	16	160%
Potatoes and sweetpotatoes	204	116	57%
Citrus fruits and tomatoes	44	98	223%
Leafy green and yellow vegetables	76	111	146%
Other vegetables and fruit	209	235	112%
Grain products	296	173	58%
Sugars and syrups	84	106	126%
Coffee, tea, and cocoa	10	19	190%

TABLE 62. TRENDS OF NUTRIENTS "AVAILABLE FOR CONSUMPTION" PER PERSON PER DAY

		1909	1949	1949/1909
Food energy	*Calories*	3510	3250	95%
Protein	*Grams*	101	94	93%
Fat	*Grams*	125	141	113%
Carbohydrate	*Grams*	487	403	83%
Calcium	*Grams*	0.86	1.05	122%
Iron	*Mgm.*	14.6	17.0	116%
Vitamin A value	*I.U.*[a]	7600	8500	112%
Thiamine	*Mgm.*	1.62	1.91	118%
Riboflavin	*Mgm.*	1.86	2.35	126%
Niacin	*Mgm.*	17.3	19.0	110%
Ascorbic acid	*Mgm.*	105.	120.	114%

[a] *I.U.* = International Units, which are also the units of the *U. S. Pharmacopeia.*

sweetpotatoes; but all modern-minded nutritionists will be glad to see the evidence of a distinct trend to higher levels of consumption of green and yellow vegetables, of citrus fruits and tomatoes, and of milk and its products other than butter.

From Table 61, but taking the items in the sequence of Table 59 (Chapter 30), we find that the trend of per capita consumption has been, for grain products and for potatoes and sweetpotatoes, distinctly downward notwithstanding the large proportion of nutrients which they furnish in proportion to their cost. Dry beans, peas, and nuts show an increase of sixty per cent in the forty years, but with amounts still so small that there is ample room for further increase of this item. Other vegetables and fruits show upward trends of twelve to over 100 per cent; while the per capita consumption of fats increased about ten per cent; that of sugars and syrups, 26 per cent; and per capita consumption of meats, fish, poultry, and eggs, considered together for our present purpose, showed a probably insignificant increase from 196 to 205 pounds. If eggs are made a separate item they show a relatively considerable increase of 31 per cent; with a probably insignificant decline (over 40 years) of consumption of two pounds a year of meats, fish, and poultry. Because meats and eggs are so similar nutritionally in what they bring the consumer, and because poultry and egg production are usually related in "the poultry enterprise," it seems best to

make one group of meat, fish, poultry, and eggs and to say that our per capita consumption from this group has been practically constant for the past forty years. In the same forty years our (U. S.) per capita consumption of milk (or of "dairy products excluding butter") is estimated to have increased about 11 per cent. This is a trend in the right direction; but is it an adequate response to the guidance offered by the growth of our nutritional knowledge during the past forty years? Perhaps the most dependable answer to this question is that afforded by the joint findings of two lines of research: (1) When full use is made of the grain products, fruits, and vegetables (the first six food-groups of Table 59 (Chapter 30) including the "direct food crops" of the agricultural economist), what nutrients will most need attention in planning the remainder of the dietary or food supply, and (2) How does milk compare with other food commodities in the efficiency and economy with which it supplies the factors which the nutritionist will most wish to emphasize at this point?

The families whose food consumption is summarized in Table 59 (Chapter 30) were taken as a random sample of the population they represented and were not so trained in nutrition and food values as to be able to make *fullest* use of the first six food-groups, yet the average use which they actually made was that of their usual and unbiased food selection. Thus used, these first six of the ten food groups *cost* 34 per cent of the total expenditure for food, and *contributed* 45 per cent of the calories, 41 per cent of the protein, 28 per cent of the calcium, 57 per cent of the iron, 58 per cent of the vitamin A value, 51 per cent of the thiamine, 28 per cent of the riboflavin, and 92 per cent of the vitamin C.

The reader may wonder that such large amounts of so many nutrients are typically furnished directly by the vegetable kingdom, even in the United States with its abundant meat and egg supply. The trend to increasing prominence of milk in the dietary or food supply may now be regarded as unquestionably established and well worthy of being accentuated.

A nutritionist recently recorded each serving of food which he ate in four years (1461 consecutive days). The total number of

servings of fruits, vegetables, and milk was over 5-fold greater than the number of servings of meat, fish, poultry, eggs, sugars, and sweets. Or, the average was, of fruits, vegetables, and milk, a total of 14 to 15 servings a day; while of meats, fish, poultry, eggs, fats, and sweets, the average total was 2 to 3 a day. Fats and sweets are counted when full sized servings, not when merely flavorings.

Obviously many families who would not wish to modernize their food habits in such thoroughgoing fashion as in the case recorded in the preceding paragraph can adopt a milder trend and presumably derive a corresponding benefit.

When nutritional guidance is not given, or is not effective, the people of higher purchasing power may indulge themselves in higher levels of intake of some foods than are conducive either to best health or to feelings of social justice. Biochemically we may not know precisely what are the optimal levels of intake of meat, or fat, or sugar; but there is good scientific ground for the view that it would be better both physiologically and psychologically if the world's meat, fat, and sugar could be more equitably distributed to, and consumed by, the world's people.

It will also be a helpful trend in this same direction if people, either voluntarily or through their governments, will reduce their market demands for grain-fed meats so that less grain will be fed to meat animals, leaving more to be used for the production of bread and milk, which are so much more economical of resources to produce than are grain-fed meats.

The Food and Agriculture Organization of the United Nations emphasizes the fact that, as a general average, seven "original calories" of feedingstuffs are required to produce one-calorie of even moderately grain-fed meat. But this "loss in processing through the animal" is much less with milk-cows than with meat animals.

Growth and diffusion of the knowledge of these facts tends to augment the trend toward fuller use of grain products and milk as human food, especially now that the Enrichment Program makes milled products less impoverished nutritionally than they were a few years or decades ago.

It is being recognized as better citizenship to make fuller use of

the grain crop directly as food for ourselves or through milk cows which "process" grain into human food much more efficiently than most meat animals do. At present (1951–52) there is a trend (which we may hope will grow) toward feeding more grass and less grain in the making of meat.

The desirable trend to increased milk production and consumption means only a moderate percentage reduction in the amount of meat produced and consumed. For most consumers readily eat more meat for pleasure whenever they have the price, whereas they often increase their milk consumption only from conviction of its importance to health and efficiency. And this conviction is reached only through the slow processes of education. Also farmers are slow to shift from meat to milk production because the latter requires a somewhat larger amount and a more thoughtful kind of labor. So increased milk production can be expected only in response to increased consumer demand. Moreover dairy farms produce meat as well as milk, and scientific research is advancing the farmer's efficiency in production of both. Hence when an educated consumer demand has substantially increased the amount of milk coming to market, it is doubtful if the market supply of meat will be noticeably different.

The acreage of fruits and vegetables can so readily be expanded that production can easily keep pace with a largely increased consumer demand for these, though the concentration of population in large cities increases the expense of transportation and makes the cost of retail distribution a serious item. Yet, even the more highly perishable fruits and vegetables, when of sufficient value per pound or ton, are now successfully transported in transcontinental carload shipments; and pre-cooling and lowered temperatures in refrigerator cars promise to reduce still further the losses incident to their transportation. Meanwhile we have learned through research in the chemistry of foods and nutrition that many of these perishable fruits and fresh vegetables, formerly regarded as luxuries, are in fact good investments for a liberal share of the consumer's food money, because of their richness in nutritional factors in which it is desirable that our dietaries be further enriched.

In order that a nutritionally guided consumer demand shall i crease the supply of the desired foods without materially increa ing prices, it is important that the science of nutrition offer practical advice to the art of dietetics in terms of foods that a crops in themselves, not merely by-products.

Planning Food Production to Meet the Needs of Nutrition and the Trends of Consumer Demand

We have approached the discussion of food economics from th viewpoint of the consumer for several reasons, among which thes are outstanding: The function of food is to nourish, and it is a food consumers that all people must get their sustenance. Ever person is a food consumer, even if he is a food producer too. Move ments for nutritional improvement of the food supply must originat either in consumer demand, or in the producer's conviction tha consumer demand is ready, or in governmental belief that a bene ficial shift in food production will have the support of publi opinion and of consumer demand. Thus the student as present an prospective consumer should realize that consumer demand mus furnish the essential support for any progressive action of govern ment or of food producers; and that in a democracy this respon sibility rests upon the citizen not only as consumer but also as factor in public opinion to support governmental food policy.

So what sounds like the business of the governmental agencie and the food producers is also a part of the information that every one will find germane to his functioning both as citizen and a consumer.

The United States Department of Agriculture regards as a very important function its annual planning of the production goal which it recommends to the farmers of the country; and, in addition to its original objective of serving the prosperity of agriculture since about 1936 this planning has increasingly been based upon the nutritionally estimated needs of our population plus each year's est mate of the amount of each crop to be sent overseas

Trend of Consumer Demand to Improve the Use of Our Food Crops and Food-production Resources

Harper of Cornell estimates that if all our edible farm products were used directly as human food they would feed fully four times our population. However, such feeding of so many people could not go on year after year, because the animal products of one year are at present produced to a considerable extent by using a large part of the previous year's grain crop as stock feed.

If all the suitable grain were used as human food, we could not at the same time support so many of all kinds of livestock in just the same manner that we now do; but by making certain moderate adjustments we could year after year furnish nutritionally excellent diets to twice as many people as our present population. This has been arrived at independently by several investigators. The present writer has studied the data sufficiently to assure himself at first hand that the estimate is well within the facts and that only moderate adjustments would be required.

In the near future, however, the best use we can make of our food crops and food-producing resources will not be simply to so arrange our food management as to export a duplicate of what we eat in this country. More good will result from a larger (and more equitably distributed) consumption of fruits, vegetables, and milk in this country; probably with substantial overseas shipments of one or more of the great staples better adapted to shipping, grain products, meats, fats. Adjustment in this direction is both nutritionally and economically sound. It is capable of working a very great improvement of nutritional well-being both at home and abroad. Such an adjustment will also mean a normal evolution of American agriculture into an increasingly better balanced pattern of production which will be more permanently profitable because of greater service to the nutritional well-being of the consumers who constitute the food "markets" both at home and abroad.

Undoubtedly a very large majority of the people of the United States are consuming total food calories about in proportion to their energy needs, and amply safe margins of total protein and of fat

above their physiological needs, but will benefit from increased consumption of fruits, vegetables, and milk. These foods are, at the same time, the ones of which the American consumer can eat more with most benefit, and the ones that are too bulky, watery, and perishable to be very practicable for large-scale export. Also Europe can, in general, produce these perishable protective foods more cheaply than she can import them; while the non-perishable or less perishable products of large-scale mechanized farming (such as the grains, and the meats and fats into which our surplus of grain is rather customarily converted), can under rational trade conditions be more economically obtained by Europe from the New World than they can be produced in older countries of dense population and high-priced land.

The reader of these pages can, as citizen, serve effectively in building a public opinion in support of such policies as will bring the benefits of our new scientific knowledge to as many people, and as promptly, as practicable; and as food consumer can also function directly through consumer-demand in bringing about the adjustments which, we now quite clearly see, can carry great nutritional benefits to the individual and family, the nation and the family of nations.

The Bureau of Human Nutrition and Home Economics has named its Commodity Summary No. 11, "Meat: variations in consumption and interrelationships with other foods."

In a week's consumption study in urban families of the United States in the spring of 1948, the meat consumed per person varied from less than 1 to more than 5 pounds.

In general, those families that used relatively large amounts of meat used smaller quantities of poultry, fish, milk, and certain vegetables and fruits than did the families using relatively small amounts of meat. Hence "low-meat" does not necessarily imply a low protein diet though it may contribute toward such a trend. In fact, both the levels of meat- and of protein-consumption have remained fairly constant in the United States in recent years.

"Also, families that used relatively large amounts of meat had diets that were superior to" (higher in) "protein, iron, thiamine, and

niacin—but inferior" (lower) "in calcium and to a lesser extent in vitamins A and C. These results indicate that for good nutrition, well-rounded diets give best results." This last sentence carries an implication which is liable to mislead many of its readers; namely, that dietaries higher in protein, iron, thiamine, and niacin are *ipso facto* "superior." Whether this implication is correct or not, depends upon *higher than what?*

Probably most readers will more or less casually assume that the "than" refers to *average* American dietaries. But this implies that average American diets are suboptimal in protein, iron, thiamine, and niacin, which implications are, in the judgment of those who seem to have best studied the subject, not supported by recent research. For protein, the optimal intake level and the average American dietary level seem to be practically the same (10 to 15 per cent of the calories as protein), and Slonaker's thorough research indicates that any materially higher level is less advantageous in the long run. The increased intakes of iron and thiamine, which present bread enrichment practices bring into the average American diet, should make it no longer suboptimal in these factors. And the same may quite reasonably appear probable for niacin when one has in mind the fact that the average American diet is helped out by the niacin produced by the bacteria of the digestive tract.

At the same time that recent research and development tend to justify reassurance regarding our intakes of protein, iron, thiamine, and niacin, other researches have shown that *best* intakes of calcium, riboflavin, and vitamins A and C are higher than the Recommended Allowances which have hitherto been regarded as ample.

Hence at the same economic levels, such shifts in the food budget as the recent advances in the science of nutrition suggest can easily increase the average American intake of calcium, riboflavin, and vitamins A and C to the improvement of the public health and the enhancement of individual efficiency, earning power, and social value.

Jennings * has made a special study of the numbers and produc-

* Jennings, R. D. 1949 Consumption of feed and relation between feed, livestock, and food at the national level. U. S. Dept. Agriculture, Circ. No. 836.

tivity of livestock (including poultry) in the United States. "Production of milk in 1945 was the highest of record," having reached this level of production and consumption by fairly steady increases over the 35-year period of 1910–1945. The impetus given by wartime demand for milk and its products both for home consumption and for shipment to Allies and Army overseas doubtless played a part in the increased production of the early forties. Had the teaching of nutrition and food values been sufficiently effective during the war years there need have been no lowering of the level of per capita production and consumption in the later forties. For we then had everything needed for continuance of the wartime level except an adequately educated consumer demand.

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Chapter 34

IMPROVEMENT OF ALREADY-NORMAL NUTRITION

Increasing Recognition of the Far-Reaching Significance of Nutrition

We have seen how McCollum has long taught that there are often important differences between the merely adequate and the optimal in nutrition; and simultaneously, J. F. Williams, doctor of medicine and professor of health education, taught that health is not merely the absence of disease, but a positive quality of life which can be built to higher levels. After a decade of such teaching Williams still remarked upon the slowness of general acceptance of this view. During more recent years, however, the constructive possibilities of nutrition have received increased recognition.

Careful scientists now speak with confidence of the hitherto unexpected potentialities of nutritional improvement of life. This is very largely due to the fact that whereas such statements were formerly regarded as matters of opinion, the most recent years have seen such developments of research methods, perfection of experimental control, increased numbers of observations, extension of experiments throughout entire lives and successive generations, and statistical scrutiny of results, that the findings have steadily acquired more and more clearly the status of objectively and conclusively established advances in the "exact science" of chemistry.

Increasingly Comprehensive Methods of Research

In the researches which have been directed particularly toward the nutritional problems of entire lifetimes and successive generations, the *experimental variables* have been of two kinds: (1) the *individual chemical factor*, element or compound as the case may

be; and (2) the *article of food* as produced by nature or agriculture and as actually consumed in daily life.

The food shortages occasioned by the World War of 1914–18, and its economic aftermath, stimulated experimentation in terms of actual articles of food. By 1930 it had been shown that a dietary made up of everyday food materials may be permanently adequate to the needs of normal nutrition, including those of growth, reproduction, and lactation, generation after generation, and yet that nutritional well-being and length of life may be improved by a more scientific adjustment of the everyday foods in the dietary.

In one such investigation, the experimental variable was the quantitative proportions in which the natural foods composing the test diets were fed. Experiments with rats showed that a mixture of 1 part dried whole milk to 5 parts ground whole wheat ("Diet A") was *adequate;* * but that an otherwise similar diet containing a higher proportion of milk ("Diet B") was *better,* as judged by each of eight criteria (Sherman and Campbell, 1924, 1928, 1930).

Simultaneously with the earliest of the Columbia experiments of this type was the work of Auden (1923) of Birmingham, England, who found that in his limited experiments, not only poorly nourished or undernourished but also "supposedly adequately nourished children," responded to the feeding of extra milk with increased gains in body weight and hemoglobin and "an improvement in bodily and mental vigor."

Also simultaneous was the work of Mann (1922–1926), whose experiments with English school boys were carried out under conditions exceptionally favorable to quantitative accuracy. In these experiments careful weighings and measurements showed that boys, who were already normally nourished at the start, made better gains in both height and weight when milk (and, in lesser degree, when watercress) was added to their accustomed diet. As the milk clearly gave better increments of gain than any of the other supplements tested, repeated comparisons of the normal diet with and without

* Rat families have repeatedly reached seventy or more generations on this Diet A (Laboratory No. 16), the limiting factor being simply that of laboratory space. Undoubtedly the diet would continue to be adequate indefinitely.

the extra milk were made. These showed undoubtedly better physical and mental growth in the boys getting the extra milk (though the diet without it was already supporting normal nutrition). Mann's experiments are discussed on pages 76–79, 99, 191–193 and 199 of *The Nutritional Improvement of Life* (Sherman, Columbia University Press).

The Lanarkshire experiment reported by Leighton and McKinlay (1930) and the later work of the "Milk Nutrition Committee" showed, by careful comparisons of large numbers of school children, that alertness and mental growth and performance, as well as growth in physique, were improved by addition of extra milk, from a starting point of already-normal nutrition.

Thus the British findings with growing children, from the intensive experimentation of Mann to the extensive studies of many thousands of school children, are consistent in showing that, starting with already-normal nutrition, both mental and physical growth and development can be improved by superior selection and proportioning of the everyday foods in the diet.

Langstroth's (1929) clinical studies of the relation between food habits and degenerative disease showed that, with adults, also, the larger the proportion of "protective" food in the diet, the smaller was the proportion of failures in the preservation of the characteristics of youth. Establishment of this fact by studies with human beings, enables us now to add still further to the significance of this finding, of the nutritional improvability of already-normal health, by comprehensive animal experimentation extending throughout entire lifetimes and successive generations of the subjects. As rats are about equally omnivorous with human beings, and very similar in those aspects of our nutrition with which we are here concerned, we work largely with them.

In the chemical laboratory of Columbia University, it was found, as already noted, that a mixture of five sixths ground whole wheat and one sixth dried whole milk, with pure table salt and distilled water, which we may call *Diet A*, was *adequate* in that it supported (in experiments with rats) normal growth and health with successful reproduction and rearing of young, generation after generation.

Yet when the proportion of milk was increased, to constitute *Diet B*, the average results were *better*. And, as we now know, *Diet B* is capable of further improvement. Such experiments, dealing with problems of improvement of already-adequate diets, and of already-normal nutrition, have now (1951–52) been carried on continuously for over twenty years and have repeatedly furnished objective evidence of the improvability of already-normal nutrition. Good judges readily agreed that better nutrition of babies contributes to the making of better babies; but acceptance of a corresponding nutritional improvement of the norm among adults (or improvement of the adult life expectation) came more slowly because it had been supposed that "longevity depends on heredity."

With the development of today's better criteria and techniques of life-time and successive-generation experiments with laboratory animals, we can now study the effects of food upon life history, both qualitatively and quantitatively, as in the work with Diets A and B mentioned above and in previous chapters.

Change in Proportions of Natural Foods

The direct comparison of Diets A and B occupied the entire natural lives of 618 experimental animals. The rats receiving Diet B made better life histories or performance records on each of eight objective criteria of positive health at these different stages of the life cycle. In the end the lengths of life attained by each sex on Diet B were enough better (higher) to make the statistical "chances" of the order of 10,000 to 1 that the differences were real and actually measured. Or the differences may be said to have about 100 times the degree of statistical conclusiveness which is considered to justify the characterization of the findings as "undoubted."

Thus it is both objectively and conclusively established that already-normal nutrition can still be nutritionally improved, and that a shift in proportions of everyday foods may measurably improve the nutritive value of the already-adequate Diet A.

In terms of articles of food, the only difference between Diets A and B was the relative proportions of wheat and milk; but the

TABLE 63. INFLUENCE OF FOOD UPON ATTAINMENT OF DEFINITE AGES BY RATS

PERCENTAGE OF LIVES LONGER THAN:	MALES		FEMALES	
	On Diet A	On Diet B	On Diet A	On Diet B
600 days	42.9	65.3	54.1	73.0
700 days	14.8	32.3	27.6	43.6
800 days	2.9	10.5	12.2	15.9
900 days	0.0	1.6	2.6	5.5
1000 days	0.0	0.0	0.5	1.2

change of this proportion made Diet B richer than Diet A in four individual (chemical) nutrients. There was a relatively small increase of protein and relatively larger increases in calcium, vitamin A, and riboflavin.

Subsequent investigation has therefore sought (among other things) to show which of these were the influential factors and what degree or level of enrichment with each is most conducive to nutritional well-being as revealed in experiments extending throughout the entire life cycles of successive generations. And as it is always to be kept in mind that there may be still-undiscovered substances essential to nutrition, comprehensive experiments were also made to test the effect of diversifying the diet with natural foods of other types. It was found that when the diet was thus diversified by adding lean beef and green snap beans there resulted somewhat more rapid growth and larger adult size, but no detectable gain in vitality or length of life. This result from a diversification of the diet (with foods well liked by the experimental animals) may be taken as evidence that no significant factor was being missed. For instance, while more milk may have meant more folic acid, it is improbable that this was a limiting factor in the experiments here discussed.

Higher Intakes of Calcium and of Vitamin A

In an extended series of experiments with diets differing in their calcium contents only to the same extent as in the above-mentioned mixtures of natural food materials (Sherman and Campbell, 1935, 1937) this increase of calcium intake (from 0.2 to 0.34–0.4 per cent

of the air-dry food mixture) resulted in slightly more rapid growth, more efficient utilization of food value whether computed in terms of calories or of protein, slightly earlier maturity, better success in reproduction and lactation, and some increase in the average length of adult life. Here the gain in longevity by the males was undoubtedly significant while that by the females was less, and if it stood alone would not be statistically convincing. But the females receiving more calcium had borne and reared more young. That they invested their extra calcium in more and better offspring and did not inherently lack the ability to profit in the same way as the males, is shown by the results of two subsequent series of experiments, as follows: (1) It was found that the above-described increment of calcium intake did increase the longevity of *unmated* females. (2) When the increase in the calcium content of the diet was more liberal there resulted both increased success in reproduction and lactation and the attainment of greater longevity by the same individual females (Campbell, Pearson, and Sherman, 1943).

With calcium intakes somewhat higher than that of Diet B (see comparison of Diets A and B above) the average lengths of adult life were increased 10.4 per cent in males and 13.8 per cent in the females as compared with Diet A. *With increased vitamin A intake,* males lived 10.1 per cent and females 12.1 per cent longer than on the Diet A described above on which rat families have repeatedly thrived for 70 to 75 generations.

Vitamin A, as we saw in Chapter 23, had been found by Batchelder and by F. L. MacLeod to yield increasingly beneficial results with increasing liberality of intake up to about four times the minimal adequate level; and an analogous four-fold zone was found by Mellanby and Green in their studies of the influence of vitamin A (or of carotene) upon ability to survive experimental infections. In an extended series of later experiments (Sherman and Campbell, 1937) it has been found that vitamin A was clearly a significant factor, along with calcium and riboflavin, in the previously reported nutritional improvement of an already-adequate diet by simple shifting of the proportions of natural foods. The data of these experiments also give interesting indications that extra liberal levels of

intake of vitamin A may have a specifically increased influence in the maintenance of high health and low death rates in the older adult experimental animals. In human affairs this would mean specifically enhanced opportunity for both the individual and the community to realize more fully upon the investment involved in one's education and years of acquirement of skill and experience.

True, the unexpectedly constructive findings of the most recent years are products of planned rather than merely random experimentation, but not all the promising possibilities have yet been tested experimentally. For example, it remains (1951–52) to be determined what life histories would result from comprehensive tests of diets carrying optimal enrichments both of calcium and of vitamin A; and from changes of diet at different points of the life-history.

Ascorbic acid (*vitamin C*) has been found by King (in experiments with guineapigs), as already explained (Chapter 17), to bring increased benefits with increase of surplus intake up to levels five to ten times as high as needed for simple prevention of scurvy. King is also studying the effects of other influences.

Riboflavin, while functioning so differently in the body, has been found, like calcium and vitamins A and C, to confer increasingly beneficial results with increasingly liberal intakes up to levels at least twice as high as that of minimal-adequacy for permanently normal nutrition. And it may prove of much significance that further increases in the riboflavin content of the diet, beyond the zone in which the individuals of the first generation show immediately tangible response, still seem to confer additional benefit upon the offspring. The most probable interpretation of these findings appears to be, that the benefit to the internal environment of the mother's body is more delicately reflected in her offspring than in such things as we yet have means to measure in her own adult organism. Fuller chemical explanation of the upper ranges of the wide zone between minimal-adequate and optimal intake of riboflavin may be expected from the combined results of (1) studies of the molecular structures of the tissue enzymes and coenzymes and the exact nature of the chemical reactions which they catalyze, and (2) determinations of riboflavin and related factors in offspring of differently fed experimental animal families at early ages by improved methods. Perhaps

a fuller understanding of these chemical factors will permit a clearer appraisal of the human significance of this nutritional improvement of an already-normal life process.

Experiments of Orr, Thomson, and Garry

Very significant too are the results obtained by Orr, Thomson, and Garry (1935) in which, in experiments continued for four generations, half the rats of a colony were fed on a dietary based on what had been found in use in a human population, while the other half received the same diet plus additional milk and green food. This enrichment of the diet resulted in: (1) greater success in the rearing of young; (2) a markedly decreased death rate, largely due to lessened incidence of, and better recovery from, an infection to which both halves of the colony were equally exposed; (3) better growth; (4) higher hemoglobin; (5) better appearance as to general condition and sleekness of coat. Hence Orr and his coworkers conclude that more milk and green vegetables in the food supply can very materially improve the physique and positive health of at least a large proportion of all the people; not simply of those suspected of malnutrition.

While, as explained in previous chapters, the zone between the minimal-adequate and optimal levels of intake is not equally wide for all nutrients, and calcium, riboflavin, and vitamins A and C were not taken at random for the comprehensive investigations here mentioned but were especially studied because of the promise of earlier work with natural foods, it is clear that the recent studies of this kind reveal previously unexpected degrees of potentiality in the improvement of already normal health and efficiency through a more scientifically guided daily use of food.

Now that the general principle of the nutritional improvability of the normal has been established with comprehensive convincingness by large numbers of full-life experiments, the way is open for further research planned with reference to the different age periods. Already it can be said with confidence that the newer knowledge of nutrition offers man a longer lease of healthier life than has before been enjoyed by any but the most fortunate few.

"Nature and Nurture"

Heredity and nutrition both have important influence upon the quality and length of life. The evidence is ample both that heredity, and that nutrition, is a significant factor; but the available research methods are so different for these two factors that the findings do not admit of a scientifically sound answer to any question as to relative importance of heredity and nutrition.

Here and elsewhere there is evidence that, in ways perhaps not yet fully understood, liberal use of protective foods may serve to diminish the incidence, or severity, or duration of other diseases as well as those which are primarily nutritional. Boudreau, Parran, and Wilder, among others, have emphasized the present-day medical view that the nutritional background and foundation afforded by the habitual use of a dietary which is thoroughly well-balanced, with reference to the newly discovered as well as longer-known factors, may greatly advance the probability of a satisfactory outcome of whatever medical measure is to be undertaken.

There is also a growing body of scientific evidence that, while physical and mental efficiency do not always go together, yet in the case of a given individual, family, or community, the superior nutritional well-being induced by taking a higher proportion of the food in the form of protective foods is favorable to the efficiency of the brain as well as of all the other organs. And we now have abundant evidence of the fact that in the well-controlled laboratory-bred colony of experimental animals, used for nutrition investigations extending throughout entire lives and successive generations, in numbers sufficiently large to permit statistical interpretation of findings, we have an instrument of research such as has not existed before, and which is throwing important light upon previously insoluble problems of the extent to which our life histories are amenable to conscious control and improvement.

That the rate or rapidity of growth does not necessarily influence the length of life or "useful" life, and, when it does, not always in the same direction, is shown by the findings summarized in Table 64.

TABLE 64. INFLUENCE OF DIET ON RATE OF GROWTH AND LENGTH OF LIFE

Rats of Columbia University Department of Chemistry Colony, 1920–1950

	RATE OF GROWTH	LENGTH OF LIFE	"USEFUL" LIFE	REFERENCE [b]
Increased proportion of milk in already adequate diet (Diet A)	Increased	Increased	Increased	Sherman and Campbell, 1924, 1928, 1930
Addition of butterfat to Diet A	Decreased	Increased	Increased	Sherman and Campbell, 1937
Addition of skim milk solids to Diet A	Increased	Increased	Increased	"
Addition of calcium to Diet A	Increased	Increased	Increased	"
Doubling the vitamin A of Diet A without adding appreciable fat	Increased	Increased	Increased	Campbell, Udiljak, Yarmolinsky, and Sherman, 1945
Again doubling vitamin A	Unchanged	Increased	Increased	Sherman and Trupp, 1949
Increase of calcium from 0.2 to 0.64% in diet	Increased	Increased	Increased	Van Duyne, et al. 1941
Diversification of diet of natural foods	Increased	Unchanged	Slightly increased	Campbell and Sherman, 1938
Increase of protein from 16% to 20% of the food	Increased	Unchanged	Slightly increased	Sherman, Campbell, and Ragan, 1949
Increase of protein from 14% to 24% of the food	Increased	Doubtful [a]	Doubtful [a]	Sherman, Pearson, and Bal, 1950

[a] The differences in these two cases were considered not statistically significant because of limited numbers of cases and wide individual variations.

[b] Cited references not included at the end of this chapter may be found at the ends of earlier appropriate chapters.

The significance of the length of life is far-reaching.

As the late President Merriam of the Carnegie Institution of Washington pointed out in his essay entitled, "Are the Days of Creation Ended?",* the direction of human evolution is now largely social, and society is a continuing organism interested in its own future. What promises to affect this future should influence our decisions from day to day and will do so more effectively with the growth of the scientific spirit which expects progress, and works for it; but meanwhile the shortness of individual lives tends to set a limit to the actual use by man of the knowledge which he has accumulated and the institutions which he has built and developed. Hence the longer term of fully efficient years which the newer chemistry of food and nutrition offers may be of exceedingly far-reaching significance to human progress.

A satisfactory life requires time. The shortness of human life, and particularly of that segment of the life cycle in which the fullest functioning is possible, often prevents the individual either from enjoying the realization of as high an aspiration, or from achieving as much in the service of others, as he could with more time. Thus in one of his annual reports, in commenting upon the deaths of colleagues, the late President Woodward of the Carnegie Institution remarked that a third of a professional or scientific man's years have usually passed by the time he has finished his formal schooling and entered his constructive life-work; then probably another third will be spent in proving to himself and to others what he is able to do, before he will be entrusted with his highest responsibilities; and so, only the last third of his years remain in which to render his fullest service to the world.

Recent chartings of the age incidence of major opportunities of presumably representative men in occupations of a scientific or related administrative or educational nature, strikingly confirm Woodward's impression that the most frequent time of attaining one's fullest opportunity is around the age of 45 to 50 years. Perhaps equally striking is the wide range of ages at which appreciable num-

* This essay is included in the book entitled, *The Living Past*, published by Scribners.

bers of men have actually found (or been admitted to) their major opportunities.

Both these facts emphasize strongly the advantages to the individual and the gains to society which may confidently be anticipated from the earlier attainment and the longer retention of the full adult capacity and efficiency of the people who receive the benefits of the new knowledge of nutrition.

Such improvements should greatly facilitate, what is now being found so important both in scientific research and in the industrial world, team-work on terms of essential equality between younger and older people, to bring into the service of a given enterprise the full advantages both of the newer training, and of the more mature experience, as well as of differing but mutually helpful points of view.

Fully to realize the importance of this, it must be kept constantly in mind that when superior nutrition increases the length of life this is only one expression of the fact that the life has been lived on a higher level of health and efficiency throughout. For the individual, it means not more years of old age but more years in the prime of life. For the nation, it means that a larger percentage of the population will be people who are mature and still of full economic, cultural, and social value.

As yet there is only a beginning of adequate appreciation of the fact that the body's "steady states" are only relatively so, and that we have power to improve both the quality and the duration of life by cultivating such food habits as help to keep the concentration of each nutrient (in the internal environment or fluid matrix) at such level as is found by research to be most conducive to well-being.

Nutritional improvement of life advances the prospect for achievement of our ideals whatever these may be.

The present is, and the near future undoubtedly will be, a period of important opportunity in the advancement of human welfare through the increase, and the diffusion, and the practical application, of nutritional knowledge. Some important things which science previously thought to be beyond its power, it is now learning to accomplish through the newer chemistry of food and nutrition.

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APPENDIX A. PROXIMATE COMPOSITION AND ENERGY VALUES OF FOODS

Food as purchased may or may not consist entirely of edible material. Meat, for example, may be purchased on the bone; or peas in the pod.

For most purposes of the present book, it seems sufficient to give the nutrients in, and the energy value of, the edible portions (E.P.) of each food listed; and it is in these terms that practically all of the values in this text are expressed. However, to give some idea of the actual nutritive value of foods as purchased, or, conversely, of the proportions of waste in various items of food as they come from the market, there is included in Table 65 a column of energy values per pound as purchased (A.P.).

Unless otherwise explained, the data of Table 65 are based on the *U. S. Department of Agriculture Handbook Number 8*, published in June 1950, in which the energy values were calculated by use of the "more specific factors" as explained in Chapter 8.

TABLE 65. PROTEIN, FAT, CARBOHYDRATE, AND ENERGY VALUES OF FOODS

FOOD	PROTEIN	FAT	CARBOHYDRATE	ENERGY VALUE	
	G./100 G.	*G./100 G.*	*G./100 G.*	*Calories /100 G.*	*Calories /Lb.*
	E.P.*	E.P.	E.P.	E.P.	A.P.*
Almonds, dried	18.6	54.1	19.6	597	2711 [a]
Apples, raw	0.3	0.4	14.9	58	232
Apricots, dried	5.2	0.4	66.9	262	1188
fresh	1.0	0.1	12.9	51	216
Asparagus, raw, fresh	2.2	0.2	3.9	21	72
Avocados	1.7	26.4	5.1	245	833
Bacon	9.1	65.0	1.1	630	2692
Bananas	1.2	0.2	23.0	88	269
Barley, pearled	8.2	1.0	78.8	349	1583
Beans, dried	21.4	1.6	61.6	338	1537
Lima, dried	20.7	1.3	61.6	333	1514
Lima, fresh	7.5	0.8	23.5	128	234 [b]
snap or string	2.4	0.2	7.7	35	143

* E.P., edible portion; A.P., as purchased.

[a] Shelled. In shell, 1386 Cal./lb.

[b] With pods. Shelled, 582 Cal./lb.

TABLE 65 (Continued). PROTEIN, FAT, CARBOHYDRATE, AND ENERGY VALUES OF FOODS

FOOD	PROTEIN	FAT	CARBOHYDRATE	ENERGY VALUE	
	G./100 G.	*G./100 G.*	*G./100 G.*	*Calories /100 G.*	*Calories /Lb.*
	E.P.	E.P.	E.P.	E. P.	A.P.
Beef, lean	19.2	12.5	0.0	195	780 [c]
Beet greens	2.0	0.3	5.6	27	93
Beets	1.6	0.1	9.6	42	142 [d]
Blackberries	1.2	1.0	12.5	57	260
Blueberries	0.6	0.6	15.1	61	279
Bluefish	20.5	4.0	0.0	124	287
Brazil nuts	14.4	65.9	11.0	646	2934 [e]
Bread, white (varies widely)	8.5	2.0	52.3	261 [h]	1184 [h]
whole wheat (Graham)	9.5	3.5	48.0	262 [h]	1188 [h]
Broccoli	3.3	0.2	5.5	29	82
Brussels sprouts	4.4	0.5	8.9	47	164
Butter	0.6	81.0	0.4 [f]	733 [h]	3251 [h]
Cabbage	1.4	0.2	5.3	24	80
Cantaloupe	0.6	0.2	4.6	20	43
Carrots	1.2	0.3	9.3	42	166 [g]
Cashew nuts, roasted	18.5	48.2	27.0	578	2622
Catsup (tomato)	2.0	0.4	24.5	98	446
Cauliflower	2.4	0.2	4.9	25	51
Celery	1.3	0.2	3.7	18	52
Chard (Swiss)	1.4	0.2	4.4	21	82
Cheese, hard (chiefly Cheddar type)	25.0	32.2	2.1	398	1806
cottage	19.2	0.8	4.3	101 [h]	459 [h]
Cherries	1.1	0.5	14.8	61	261
Chestnuts	2.8	1.5	41.5	191 [h]	866 [h, i]
Chicken, total edible part	20.2	7.2	0.0	151	378 [j]
Chocolate, bitter	(5.5) [k]	52.9	29.2	501	2275
sweet, plain	(2.)	29.8	62.7	471	2141
sweet, milk	(6.)	33.5	55.7	503	2283

[c] Includes some bone.
[d] Without tops. With tops, 101 Cal./lb.
[e] Shelled. In shell, 1468 Cal./lb.
[f] Includes both the lactose and the lactic acid of that part of the buttermilk which the butter retains.
[g] Without tops. With tops, 119 Cal./lb.
[h] Atwater factors.
[i] Shelled. In shell, 699 Cal./lb.
[j] Dressed.
[k] Figures in parentheses indicate imputed value.

TABLE 65 (Continued). PROTEIN, FAT, CARBOHYDRATE, AND ENERGY VALUES OF FOODS

FOOD	PROTEIN	FAT	CARBOHYDRATE	ENERGY VALUE	
	G./100 G.	*G./100 G.*	*G./100 G.*	*Calories /100 G.*	*Calories /Lb.*
	E.P.	E.P.	E.P.	E.P.	A.P.
Clams	12.8	1.4	3.4	81	129 [l]
Cocoa, dry	(8.)	23.8	48.9	293	1331
Coconut, dried, shredded, sweetened	3.6	39.1	53.2	556	2525
fresh	3.4	34.7	14.0	359	865
milk	0.3	0.4	5.0	25	
Codfish	16.5	0.4	0.0	74	306 [m]
Collards	3.9	0.6	7.2	40	82
Corn (maize), whole	10.0	4.3	73.4	372 [h]	1689 [h]
ground, dry	9.2	3.9	73.7	355	1611
sweet	3.7	1.2	20.5	92	160 [n]
Cornflakes	8.1	0.4	85.0	385	1748
Cornmeal, degermed	7.9	1.2	78.4	363	1650
Crackers, Graham	8.0	10.0	74.3	393	1785
saltines	9.2	11.8	71.1	431	1955
soda, plain	9.6	9.6	72.7	420	1909
Cranberries	0.4	0.7	11.3	48	218
Cream, thick (whipping)	2.3	35.0	3.2	330	1497
thin (coffee)	2.9	20.0	4.0	204	925
Cucumbers	0.7	0.1	2.7	12	39
Currants, dried	2.3	0.5	71.2	298 [h]	1353 [h]
fresh, red	1.2	0.2	13.6	55	241
Dandelion greens	2.7	0.7	8.8	44	200
Dates, dried	2.2	0.6	75.4	284	1121 [o]
Duck	16.0	28.6	(0)	321 [h]	?
Eggplant	1.1	0.2	5.5	24	95
Eggs	12.8	11.5	0.7	162	655
Egg white	10.8	0.0	0.8	50	227
Egg yolk	16.3	31.9	0.7	361	1640
Endive and Escarole	1.6	0.2	4.0	20	47
Farina	10.9	0.8	77.4	370	1678
Figs, dried	4.0	1.2	68.4	270	1224
fresh	1.4	0.4	19.6	79	357
Flounder	14.9	0.5	0	68	121 [p]
Flour, patent	10.5	1.0	76.1	364	1654
whole wheat	13.3	2.0	71.0	333	1511

[l] For long clams with shells; round clams with shells, 63 Cal./lb.
[m] Steaks, includes bone.
[n] With husks.
[o] With pits.
[p] Whole.

TABLE 65 (Continued). PROTEIN, FAT, CARBOHYDRATE, AND ENERGY VALUES OF FOODS

FOOD	PROTEIN	FAT	CARBOHYDRATE	ENERGY VALUE	
	G./100 G.	*G./100 G.*	*G./100 G.*	*Calories /100 G.*	*Calories /Lb.*
	E.P.	E.P.	E.P.	E.P.	A.P.
Grapefruit	0.5	0.2	10.1	40	119
juice, fresh or canned	0.5	0.1	9.2	36	162
Grapes	1.4	1.4	14.9	70	248
Guavas	1.0	0.6	17.1	70	276
Haddock	18.2	0.1	0.0	79	171 [q]
Halibut	18.6	5.2	0.0	126	464 [m]
Ham	15.2	31.0	0.0	344	1345 [c]
Hazelnuts	12.7	60.9	17.7	670 [h]	3039 [h, r]
Heart, beef	16.9	3.7	0.7	108	491
Herring (Atlantic), raw	18.3	12.5	0.0	191	443 [p]
Honey (strained or extracted)	0.3	0.0	79.5	294	1333
Ice cream, plain	4.0	12.5	20.6	207	938
Jams, marmalades, preserves	0.5	0.3	70.8	278	1263
Jellies	0.2	0.0	65.0	252	1145
Kale	3.9	0.6	7.2	40	117
Kidneys (beef)	15.0	8.1	0.9	141	639
Kohlrabi	2.1	0.1	6.7	30	73
Lamb	14.9	32.4	0.0	356	1228 [c]
Lard	0.0	100.0	0.0	902	4095
Lemons	0.9	0.6	8.7	32	88
juice, fresh or canned	0.4	0.2	7.7	24	108
Lentils, dry	25.0	1.0	59.5	337	1531
Lettuce	1.2	0.2	2.9	15	47
Limes	0.8	0.1	12 3	37	126
Liver (beef)	19.7	3.2	6.0	136	618
Lobster	16.2	1.9	0.5	88	144 [p]
Macaroni, dry	12.8	1.4	76.5	377	1712
Mackerel, Atlantic	18.7	12.0	0.0	188	457 [q]
Mango	0.7	0.2	17.2	66	198
Margarine	0.6	81.0	0.4	720	3269
Marmalade, *see* Jams					
Mayonnaise, comm'l, plain	1.1	36.8	13.9	384	1743
Milk, cows', whole	3.5	3.9	4.9	68	309
non-fat, skim	3.5	0.1	5.1	36	162

[q] Skin, bones, and entrails included.
[r] Shelled. In shell, 1429 Cal./lb.

TABLE 65 (Continued). PROTEIN, FAT, CARBOHYDRATE, AND ENERGY VALUES OF FOODS

FOOD	PROTEIN	FAT	CARBOHYDRATE	ENERGY VALUE	
	G./100 G.	*G./100 G.*	*G./100 G.*	*Calories /100 G.*	*Calories /Lb.*
	E.P.	E.P.	E.P.	E.P.	A.P.
condensed, sweetened	8.1	8.4	54.8	320	1455
evaporated, unsweetened	7.0	7.9	9.9	138	625
malted, dry	14.6	8.5	70.7	407	1850
skimmed, dried	35.6	1.0	52.0	362	1643
whole, dried	25.8	26.7	38.0	492	2233
Milk, goats'	3.3	4.0	4.6	67	305
Molasses, cane					
1st extraction, or light	—	—	65.0	252	1142
2nd extraction, or medium	—	—	60.0	232	1054
3rd extraction, or blackstrap	—	—	55.0	213	966
Barbados	—	—	70.0	271	1230
Muffins	8.0	8.4	42.1	280	1269
Mushrooms	2.4	0.3	4.0	16	66
Mustard greens	2.3	0.3	4.0	22	74
Noodles (containing egg)	12.6	3.4	73.2	381	1729
Oatmeal or rolled oats	14.2	7.4	68.2	390	1770
Oils, salad	0.0	100.0	0.0	884	4013
Okra	1.8	0.2	7.4	32	130
Oleomargarine, *see* Margarine					
Olives, green	1.5	13.5	4.0	132	504
ripe	1.8	21.0	2.6	191	728
Onions, mature	1.4	0.2	10.3	45	193
young, green	1.0	0.2	10.6	45	84
Oranges	0.9	0.2	11.2	45	147
juice	0.8	0.2	11.0	44	199
Oysters, meat only, raw	9.8	2.1	5.6	84	380
Parsley	3.7	1.0	9.0	50	225
Parsnips	1.5	0.5	18.2	78	277
Peaches	0.5	0.1	12.0	46	183
Peanuts, Virginia type, roasted	26.9	44.2	23.6	559	2540 [a]
Peanut butter	26.1	47.8	21.0	576	2615
Pears	0.7	0.4	15.8	63	236

[a] Shelled. In shell, 1829 Cal./lb.

TABLE 65 (Continued). PROTEIN, FAT, CARBOHYDRATE, AND ENERGY VALUES OF FOODS

FOOD	PROTEIN	FAT	CARBOHYDRATE	ENERGY VALUE	
	G./100 G.	*G./100 G.*	*G./100 G.*	*Calories /100 G.*	*Calories /Lb.*
	E.P.	E.P.	E.P.	E.P.	A.P.
Peas, green, young, fresh	6.7	0.4	17.7	98	201 [t]
Peas, mature, dry seeds	23.8	1.4	60.2	339	1541
Pecans	9.4	73.0	13.0	696	3162 [u]
Peppers, green	1.2	0.2	5.7	25	95
Persimmons	0.8	0.4	20.0	78	270
Pickles, dill, cucumber	0.7	0.2	2.1	11	49
Pie, apple	2.1	9.5	39.5	246	1115
Pineapple, canned	0.4	0.1	21.1	78	355
fresh	0.4	0.2	13.7	52	126
juice, canned	0.3	0.1	13.0	49	221
Plums (except prunes)	0.7	0.2	12.9	50	218
Pork, lean cuts	14.5	32.7	0.0	357	1346
Potatoes, raw	2.0	0.1	19.1	83	318
Prunes, dried	2.3	0.6	71.0	268	1036
Pumpkin	1.2	0.2	7.3	31	96
Radishes	1.2	0.1	4.2	20	45
Raisins	2.3	0.5	71.2	268	1218
Raspberries	1.2	0.4	13.8	57	259
Rhubarb	0.5	0.1	3.8	16	50
Rice, brown	7.5	1.7	77.7	360	1634
white	7.6	0.3	79.4	362	1644
Rutabaga	1.1	0.1	8.9	38	147
Rye meal or whole grain	12.1	1.7	73.4	321	1456
Salmon, Chinook or king	17.4	16.5	0.0	223	902 [v]
Sardines, canned in oil	21.1	27.0	1.0	338	1534
Sauerkraut, solids	1.4	0.3	4.4	22	75
Sausage, Bologna	14.8	15.9	3.6	221	1005
frankfurter	14.2	20.5	2.7	257	1166
pork	10.8	44.8	0.0	450	2044
Scallops, edible muscle	14.8	0.1	3.4	78	355
Shad	18.7	9.8	0.0	168	367 [p]
Shrimp, canned	26.8	1.4	0.0	127	577
Sirup, table blends	(υ)	(0)	(74.0)	286	1300
Soup, vegetable, ready to serve	1.7	0.7	5.8	33	149

[t] With pods. Shelled, 448 Cal./lb.
[u] Shelled. In shell, 1644 Cal./lb.
[v] Bones and skin included.

TABLE 65 (Continued). PROTEIN, FAT, CARBOHYDRATE, AND ENERGY VALUES OF FOODS

FOOD	PROTEIN	FAT	CARBOHYDRATE	ENERGY VALUE	
	G./100 G.	*G./100 G.*	*G./100 G.*	*Calories /100 G.*	*Calories /Lb.*
	E. P.	E. P.	E. P.	E. P.	A. P.
Soybeans, whole, mature dried	34.9	18.1	34.8	331	1503
Soybean flour, flakes, grits, low fat	44.7	1.1	37.7	228	1034
medium fat	42.5	6.5	37.2	264	1200
full fat	35.9	20.6	29.9	347	1576
Spinach	2.3	0.3	3.2	20	73
Squash, summer	0.6	0.1	3.9	16	71
winter	1.5	0.3	8.8	38	126
Strawberries	0.8	0.5	8.3	37	160
Sugar, granulated	(0)	(0)	99.5	385	1748
brown	(0)	(0)	95.5	370	1678
maple	—	—	(90.0)	348	1583
Sweetpotatoes	1.8	0.7	27.9	123	480
Tapioca	0.6	0.2	86.4	360	1634
Tomatoes	1.0	0.3	4.0	20	81
Tongue, medium fat	16.4	15.	0.4	207	892
Tuna fish, canned	23.8	20.9	0.0	290	1318
Turkey	20.1	20.2	0.0	268	815 [j]
Turnip greens	2.9	0.4	5.4	30	135
Turnips (white)	1.1	0.2	7.1	32	96
Veal, lean	19.7	8.0	0.0	156	547
Vinegar	0.	—	(5.0)	12	56
Walnuts	15.0	64.4	15.6	654	2972 [w]
Watercress, leaves and stems	1.7	0.3	3.3	18	84
Watermelon	0.5	0.2	6.9	28	58
Wheat, whole	11.1	1.7	75.5	362 [h]	1644 [h]
shredded	10.1	2.5	80.1	360	1635
Whey, fluid	0.9	0.3	5.1	26	120
Yeast, baker's, compressed	(10.6)	0.4	13.0	86	388
brewer's, dried	(36.9)	1.6	37.4	273	1239

[w] Shelled. In shell, 1335 Cal./lb.

APPENDIX B. MINERAL ELEMENTS IN FOODS

Table 66 gives for the chief articles of food, the *general average* values, expressed as *milligrams per 100 grams of edible portion*, for calcium, magnesium, potassium, sodium, phosphorus, chlorine, sulfur, and iron. These averages take account of all available evidence, including data determined in the Columbia University chemical laboratories and not published separately. As these averages have been revised for successive editions of this book it has been found that with increasing numbers of determinations of a given element in a given food, the average tends to become stabilized. Fair degrees of stability and thus of *validity as general averages* now appear to have been reached, so that for *general* computations the data of Table 66 may be used with reasonable confidence, particularly when one is dealing with food supplies obtained in markets that draw from widespread sources. Individual samples may, however, vary considerably from the general average, as has been explained in Chapter 31.

TABLE 66. AMOUNTS OF SOME MINERAL ELEMENTS IN MILLIGRAMS PER 100 GRAMS EDIBLE PORTION OF FOODS

FOOD	CALCIUM	MAGNESIUM	POTASSIUM	SODIUM	PHOSPHORUS	CHLORINE	SULFUR	IRON
Almonds	254	252	747	3	475	20	150	4.4
Apples	6	6	111	2	11	4	5	.3
Apricots, dried	92	65	1790	34	115	35	? †	5.9
fresh	15	9	311	1	24	2	6	.5
Asparagus	21	12	187	3	57	36	46	1.2
Avocado	16	41	679	3	46	16	37	1.2
Bacon [a]	13	13	214	820 [a]	108	1251 [a]	152	?
Bananas	11	31	378	4	28	125	12	0.6
Barley, entire	75	171	485	?	373	?	143	5.1
pearled	16	37	126	3	189	104	116	(2.)
Beans, dried	148	159	1213	1	437	35	237	(8.)
Lima, dried	68	181	1727	?	381	31	178	7.5
fresh	31	*	?	1	112	33	25	2.3
snap or string	65	27	251	3	44	33	30	1.1
Beef, lean	11	22	333	65	204	76	230	3.0
Beet greens	118 [b]	113	541	135	41	*	*	3.2
Beets	22	23	331	57	39	61	17	1.0
Blackberries, seeds included	32	24	174	2	32	15	17	.9
Blackberries, seeds removed	17	*	*	2	19	7	8	(.9)
Blueberries	16	10	73	1	?	8	11	.8
Bluefish	23	31	315	68	243	76	241	.6
Brazil nuts	?	225	601	2	625	61	198	3.4
Bread, white [c]	(79) [d]	30	109	511 [a]	92	701 [a]	54	.6 **
whole wheat [c]	(96) [d]	74	224	671 [a]	263	831 [a]	102	2.2
Broccoli	140	24	336	10	76	?	137	1.3
Brussels sprouts	36	28	332	9	92	11	131	1.3
Butter	16	1	14	(220) [a]	16	(330) [a]	9	0.0
Cabbage, headed	46	12	294	5	31	39	67	0.5
loose leafed	429	34	402	18	72	108	(70)	1.8

Cantaloupe	17	17	249	15	16	40	15	.4
Carrots	39	16	334	51	37	55	21	.8
Cashew nuts	46	267	560	13	480	*	*	5.0
Cauliflower	22	20	313	24	62	31	85	1.1
Celery	60	25	290	96	40	137	22	.7
Chard	83 [b]	53	349	85	45	39	124	3.5
Cheese, hard	873	42	131	[a]	610	[a]	218	(1.)
cottage	82	*	*	*	263	*	*	(1.)
Cherries	18	14	246	3	20	3	8	.5
Chestnuts	34	42	489	7	90	11	48	.8
Chicken (fowl)	14	27	343	75	200	79	252	1.5
Chocolate, bitter	98	292	830	4	446	?	196	5.6
plain, sweet	(63)	107	327	?	?	?	32	2.8
milk	216	58	418	?	283	151	67	?
Clams	96	89	172	603	(139)	1065	219	(7)
Cocoa	125	420	900	?	709	51	203	?
Coconut, dried	43	77	719	22	191	225	76	3.6
fresh	?	39	363	39	98	122	32	2.0
"milk"	24	?	?	?	29	190	32	.1
Codfish	10	22	339	75 [a]	194	150 [a]	203	1.2
Collards	250	55	425	*	74	*	*	1.6
Conch	89	246	*	*	112	*	315	1.2

N.B. Data enclosed in parentheses are based on evidence either less consistent or less direct than in the majority of cases.

* Doubtless present but quantitative data have not been found.

? Data too few or too variable to average.

‡ Varies with processing. Higher when sulfured.

** According to Federal standards for enrichment, the following minimum values are required:

Bread, white	1.8 mg./100 grams
Cornmeal	2.9 mg. 100 grams
Farina	1.3 mg./100 grams
Flour, white	2.9 mg. 100 grams

[a] Varies with amount of added salt.

[b] May not be available because of presence of oxalic acid.

[c] Uncertain because of varying methods of breadmaking.

[d] Varies with amount of milk solids, conditioners, "yeast food," etc., used in breadmaking.

TABLE 66 (Continued). AMOUNTS OF SOME MINERAL ELEMENTS IN MILLIGRAMS PER 100 GRAMS EDIBLE PORTION OF FOODS

FOOD	CALCIUM	MAGNESIUM	POTASSIUM	SODIUM	PHOSPHORUS	CHLORINE	SULFUR	IRON
Corn (maize), entire	(20)	(120)	(350)	1	302	45	151	3.6
meal	16	86	349	?	174	?	127	2.7
"sweetcorn"	9	38	?	1	120	14	46	.5
Cranberries	14	7	78	3	11	5	7	.6
Cream, "light" (thin)	97	(10)	(91)	(30)	77	(80)	30	.1
"heavy or whipping" (thick)	78	(10)	(91)	(30)	61	(80)	30	.0
Cucumbers, seeds included	?	9	?	7	21	30	12	.3
Cucumbers, seeds removed	6	*	*	*	18	*	*	.3
Currants, dried	75	30	(550)	18	138	29	10 †	2.7
fresh	36	15	(250)	2	33	13	29	.9
Currant juice	16	10	185	(6)	13	4	5	*
Dandelion	150	36	446	76	43	99	17	3.1
Dates	72	65	675	1	60	?	65	2.1
Eggplant	15	15	229	2	27	47	21	.5
Eggs	54	13	138	137	210	120	197	2.7
Egg white	11	11	154	170	17	161	208	.1
Egg yolk	157	16	118	56	538	124	194	7.2
Endive and Escarole	79	13	387	10	40	71	32	(2)
Farina	21	25	86	2	112	?	155	1.0
Figs, dried	(200)	82	(900)	(30)	(100)	?	69	3.0
fresh	54	21	(290)	2	32	14	12	.6
Fish [e]								
Flounder	40	25	311	68	195	151	217	.8
Flour, Graham or entire wheat	41	125	324	3	(350)	38	124	3.9
white (wheat)	16	37	128	2	100	49	109	1.3
Grapefruit	22	10	198	2	18	3	8	.3
juice	10	7	129	2	15	2	5	.3
Grapes, seeds removed	17	7	195	2	21	2	9	.6
Haddock	23	26	314	? [a]	197	? [a]	238	.7

Halibut	13	24	340	56	211	88	212	.7
Ham, medium fat	10	20	?	1603 [a]	136	2469 [a]	225	2.5
Hazelnuts	287	140	618	19	354	67	198	.1
Heart	10	35	257	80	198	125	296	5.3
Herring	40	26	303	100	241	122	202	.9
Honey	5	6	?	9	12	25	5	.9
Kale	225	37	401	56	67	122	119	2.7
Kidney	14	16	250	215	237	220	156	8
Kohlrabi	46	37	337	10	50	53	50	.6
Lamb (mutton), lean	10	24	327	90	(200)	85	211	2.5
Lemon (or juice)	20	9	148	2	12	4	8	.3
Lettuce, headed	32	13	267	14	29	64	13	1.1
all other	70	13	313	14	28	79	23	1.6
Liver	8	22	296	91	373	101	251	12.1
Macaroni	22	36	162	12	145	52	136	1.5
Mackerel	10	33	418	? [a]	250	? [a]	197	1.0
Meat [f]								
Milk, cows'	118	12	143	51	93	106	34	0.1
Molasses, 1st extraction or light	165	(46)	(917)	(15)	45	(224)	(32)	4.3
2nd extraction or medium	290	(81)	(1063)	(37)	69	(576)	*	6.0
Mushrooms	9	16	494	7	115	21	32	0.8
Mustard greens	(200)	5	394	34	60	*	?	?
Mutton, see Lamb								
Oatmeal (oats)	53	145	431	2	405	49	199	5.
Okra, seeds included	80	38	220	1	62	*	14	0.8
seeds removed	75	43	*	*	53	*	*	(.8)
Olives [g]	87	12	?	1668 [a]	17	1877 [a]	32	1.6

[e] Average fish is estimated to contain per 100 grams of protein as follows: 0.109 gram Ca; 0.133 gram Mg; 1.671 grams K; 1.148 grams P; 1.119 grams S; 0.0055 gram Fe.

[f] Average meat is estimated to contain per 100 grams protein as follows: 0.058 gram Ca; 0.118 gram Mg; 1.694 grams K; 1.078 grams P; 1.146 grams S; 0.0150 gram Fe.

[g] Pickled in brine.

TABLE 66 (Continued). AMOUNTS OF SOME MINERAL ELEMENTS IN MILLIGRAMS PER 100 GRAMS EDIBLE PORTION OF FOODS

FOOD	CALCIUM	MAGNESIUM	POTASSIUM	SODIUM	PHOSPHORUS	CHLORINE	SULFUR	IRON
Onions	36	16	162	7	34	24	69	0.5
Oranges	38	11	185	2	23	6	10	0.4
juice	19	10	166	1	16	4	6	0.2
Oysters	70	39	?	73	150	628	180	6.
Parsley	243	41	809	30	77	141	190	8.
Parsnips	57	29	465	8	80	35	26	0.7
Peaches	8	11	212	4	22	5	7	0.4
Peanuts	74	167	632	4	393	17	226	1.9
Peanut butter	36	178	757	(120)	390	?	225	1.9
Pears	13	9	127	3	16	4	7	0.3
Peas, dried	72	140	979	35	388	44	196	5.2
fresh	26	33	315	4	128	33	51	2.0
Pecans	80	151	603	1	324	50	113	2.4
Peppers, green	9	12	177	1	25	19	19	0.6
Persimmons	6	9	316	6	26	2	5	0.3
Pineapple	16	11	218	2	11	46	6	0.4
Plums	17	11	198	4	20	2	5	0.5
Pork, medium lean	9	24	300	68	198	34	190	1.8
10% protein	6	12	169	42	108	38	115	1.5
Potatoes	11	27	496	5	56	35	29	0.7
Prunes, dried	62	44	700	6	93	9	28 †	3.9
Pumpkin	21	12	457	1	37	?	13	0.8
Radishes	37	15	285	14	31	37	31	1.0
Raisins	60	27	803	31	110	24	42 †	3.3
Raspberries, seeds included	28	22	178	2	37	22	18	0.9
seeds removed	24	20	(140)	2	25	*	(10)	(0.9)
Rhubarb	? b	16	286	2	25	53	8	0.5
Rice, brown	(50)	106	275	?	303	23	?	?
white	9	28	92	4	92	6	106	0.8

Rutabaga	55	15	255	5	41	31	36	0.5
Rye, entire	61	155	453	?	376	?	146	4.8
flour	22	65	(405)	1	253	55	134	1.3
Salmon	[h]	29	331	53 [a]	289	88 [a]	226	0.9
Sardines, canned	(380)	41	393	614 [a]	683	1200 [a]	283	4.0
Shrimps	115	74	312	140 [a]	210	? [a]	339	2.0
Sirup, table blends	(46)	(10)	?	63	(16)	74	?	(4.1)
Soybeans, mature, dry	227	255	1834	4	586	24	323	8.0
Soybean flour	250	*	1700	1	583	(24)	(290)	(2.7)
Spinach	81 [b]	52	502	84	49	65	27	3.0
Squash, summer, seeds removed	15	8	150	1	15	*	*	.4
winter, seeds removed	19	24	407	1	42	*	*	1.1
Strawberries	28	12	149	2	27	11	12	.8
Sweetpotatoes	30	24	373	13	49	85	26	.8
Tapioca	12	2	20	4	12	16	4	(1.)
Tomatoes, seeds included	9	12	258	4	27	(37)	13	0.6
seeds removed	6	10	229	4	20	(40)	8	0.6
juice	7	10	269	15 [a]	15	55 [a]	5	(0.4)
Tongue	9	22	225	86	187	?	(194)	(2.8)
Turkey	23	28	332	?	320	123	234	3.8
Turnip greens	260	19	374	9	58	92	54	2.4
Turnips, white	44	18	333	50	34	52	58	0.5
Veal, medium-lean	11	23	345	?	221	77	203	2.4
Vinegar	16	11	?	1	15	47	18	0.4
Walnuts	83	134	503	2	380	14	146	2.1
Watercress	168	28	301	?	46	109	147	2.6
Watermelon	7	10	121	1	12	8	9	0.2
Wheat, entire	54	147	430	12	374	49	171	5.7
Wine (average)	9	7	104	7	15	2	15	(0.3)

[h] The calcium content of the edible flesh of fresh salmon, carefully freed from bone, averages about 0.015 per cent. Canned salmon, however, ordinarily includes the bone, and according to the U. S. Department of Commerce, this should be reckoned as consumable and nutritionally available. With bone thus included, the calcium content of salmon averages about 0.194 per cent.

APPENDIX C. VITAMIN VALUES OF FOODS

Table 67 gives vitamin C, thiamine, riboflavin, and vitamin A values for the chief food sources of these vitamins. For the purposes of this textbook, it seems that to give single average values would be premature because there is not yet a sufficient consensus of opinion as to accuracy of methods of vitamin assay to permit of our regarding present averages as sufficiently stabilized in the majority of cases. Besides the variability of laboratory methods used in measuring vitamin values, there are also relatively wide variations of actual values as explained in Chapter 31. Table 67, therefore, instead of general averages that might in many cases be tentative and possibly mislead, gives *ranges within which it appears probable that the average when sufficiently established will be found.* This is believed to give a much truer and more serviceable picture of the normal variation than is given by the recording of the maximum and minimum of published data when these are so likely to be influenced by faulty methods or other errors.

Where sufficient numbers of samples have been studied by sufficiently trustworthy methods to justify statistical interpretation, the range is so set as to make the chances about 100:1 that the ultimate mean of similar studies will fall within the range here given.

In this table the giving of a single figure indicates insufficient evidence to permit an estimate of the range. Data enclosed in parentheses are based on evidence either less consistent or less direct than in the majority of cases.

TABLE 67. VITAMIN VALUES OF FRESH RAW FOODS PER 100 GRAMS OF EDIBLE PORTION

FOOD	VITAMIN C	THIAMINE	RIBOFLAVIN	VITAMIN A VALUE
	Mg.	*Mg.*	*Mg.*	*I.U.*
Almonds	——	.12–.30	.50–.80	?
Apples	5–8	.02–.04	.02–.05	40–100
Asparagus	25–50	.11–.20	.17	300–1000
Avocado	2–16	.10–.20	.14	110–290
Banana	6–10	.04–.10	.04–.08	160–450

TABLE 67 (Continued). VITAMIN VALUES OF FRESH RAW FOODS PER 100 GRAMS OF EDIBLE PORTION

FOOD	VITAMIN C	THIAMINE	RIBOFLAVIN	VITAMIN A VALUE
	Mg.	*Mg.*	*Mg.*	*I.U.*
Barley, entire	—	.50–.65	.09–.14	71
pearled	—	(.18)	.08	—
Beans, dried	—	.40–.70	.37	—
Lima, dried	—	.40–.70	.18–.79	—
fresh	30–50	.15–.35	.10–.25	270–500
snap or string	17–20	.06–.09	.10–.15	600–1000
Beef, lean muscle	—	.08–.16	.15–.30	10–50
Beets	3–10	.02–.09	.02–.12	20–80
Bran	—	.23–.70	.39	—
Bread, white				
(varies widely)	—	.05–.07 *	.07–.13 *	—
whole wheat	—	.30–.40	.10–.16	—
Broccoli	108–122	.08–.10	.15–.30	3000–9000
Brussels sprouts	20–100	.08–.17	.17	300–500
Butter	—	—	—	3300–5000
Cabbage, headed	29–52	.04–.08	.05–.30	30–80
loose leaf				880
Cantaloupe	26–35	.03–.08	.04–.08	400–3500
Carrots	5–7	.05–.10	.05–.09	3000–12,000
Cauliflower	69–83	.10–.15	.10–.22	35–90
Celery, stalk	6–8	.02–.05	.03–.06	0–50
Chard	10–40	.06–.09	.07–.13	(10,000) **
Cheese, Cheddar type	—	.02–.07	.50–1.00	1400–4000
Cherries	8–10	.05	(.02)	200–700
Chicken	—	.07–.11	.10–.20	—
Collards	30–100	.10–.25	.25	(10,000) **
Corn (maize), mature	—	.44–.49	.13–.16	[a]
meal	—	.05–.30 [b]	.05–.11 [b]	[a]
sweet	8–12	.12–.15	.10–.17	[a]
Cranberries	10–13	(.03)	(.04)	10–40
Cream, thick (whipping)	1–1.5	.02–.04	.12–.15	1400–2500
thin (coffee)	1–2	.03–.04	.14–.18	800–1500
Cucumbers	2–13	.03–.09	.04–.15	15–50
Dandelion	5–40	.15–.23	.14	(10,000) **
Dates, dried	—	.06–.10	.05	60–300
Eggplant	4–9	.04–.10	.03–.08	20–100

* Current (1951) Federal standards for *enriched* bread require a minimum of .240 mg. thiamine and .150 mg. riboflavin per 100 grams bread.

** Data for "Greens" used for Chard, Dandelion, Escarole, Kale, Mustard greens, Spinach, and Turnip greens. (See Chap. 23, pp. 482–483.)

[a] Significant vitamin A values in the yellow, but not in the white varieties. Yellow corn meal reported to have 300–750 International Units per 100 grams.

[b] "Enriched" corn meal and "enriched" white flour contain a minimum of .44 mg. thiamine and .26 mg. riboflavin per 100 grams, according to current (1951) government regulations.

TABLE 67 (Continued). VITAMIN VALUES OF FRESH RAW FOODS PER 100 GRAMS OF EDIBLE PORTION

FOOD	VITAMIN C	THIAMINE	RIBOFLAVIN	VITAMIN A VALUE
Eggs	—	.08–.12	.30–.50	1000–2000
Egg white	—	trace	.15–.30	trace
Egg yolk	—	.25–.35	.30–.60	3000–4000
Endive and Escarole	6–15	.09	.12–.40	(10,000) **, c
Figs, dried	—	.08–.18	.08–.13	50–90
fresh	2	.05–.10	.06	60–90
Flour, white	—	.06–.10 b	.03–.05 b	—
whole wheat	—	.50–.60	.10–.20	—
Grapefruit (or juice)	37–41	.04–.10	.02–.10	21
Grapes	3–5	.03–.06	.01–.06	20–80
Guavas	60–300	.04–.08	.09	200
Ham, lean	—	.60–1.20	.19–.30	trace
Kale	115–136	.10–.20	.25–.40	(10,000) **
Kidney	trace	.40–.50	1.70–2.20	500–1200
Kohlrabi	40–80	.04–.07	(.03)	trace
Lamb (and Mutton)	—	.15–.30	.19–.29	trace
Lemon (or juice)	50–56	.03–.09	(.02)	trace
Lettuce, headed	8–18	.04–.10	.04–.08	70–700
loose leaf			.10–.20	700–7000
Liver	1–36	.25–.40	2.00–4.00	10,000–40,000
Milk, cows'	1–2.2	.03–.04	.17–.25	160–225
evaporated	trace	.05–.08	.33–.38	300–450
dry, whole	(5–10)	.30–.32	1.30–1.60	1300–1800
Mustard greens	50–100	.09	.20–.38	(10,000) **
Oatmeal (oats)	—	.54–.82	.12–.16	—
Okra	26–34	.13	.07–.45	300–800
Onions	8–24	.02–.10	.02–.06	trace
Orange (or juice)	49–56	.07–.09	.02–.09	50–400
Oysters	3	.15–.30	.20–.50	150–350
Parsnips	18–24	.08–.19	.06–.12	trace
Peaches, white	6–9	.02–.07	.04–.05	0–100
yellow				1000–2000
Peanuts, roasted	—	(.30)	.10–.35	?
Peanut butter		(.15)		
Pears	3–5	.02–.10	.02–.15	10–20
Peas, dried	—	.54–.93	.15–.37	370
fresh, green	22–26	.34–.42	.10–.30	600–1300
Pecans	—	.15–.72	.11–.30	50–200
Peppers, green	90–150	.02–.07	.05	600–5000
Pineapple, fresh	13–25	.08–.13	.02–.08	40–130
Plums	4–7	.05–.20	.03–.04	350
Pork, lean	—	.68–1.15	.20–.40	trace
Potatoes, white	14–20	.11–.13	.03–.05	20–50
Poultry, dark meat	—	(.11)	.22–.32	—
light meat			.06–.10	

c Bleached varieties have little or no vitamin A value.

TABLE 67 (Continued). VITAMIN VALUES OF FRESH RAW FOODS PER 100 GRAMS OF EDIBLE PORTION

FOOD	VITAMIN C	THIAMINE	RIBOFLAVIN	VITAMIN A VALUE
Prunes, dried	0–8	.10–.20	.05–.35	400–2400
Pumpkin	5–10	(.05)	.05–.09	(2000–4000)
Radishes	12–24	.03–.10	.02–.04	30
Raisins	—	.10–.20	.08–.13	10–100
Raspberries	8–75	.02–.03	.07	(320)
Rhubarb	9–24	.01–.03	—	30–100
Rice, brown	—	.28–.32	.05–.08	0–100
white	—	.04–.07	.03	—
"converted"	—	.20–.25	.05	—
Salmon	—	.13	.16–.25	20–600
Soybeans, mature	—	.90–1.14	.31	110
Spinach	15–60	.10–.14	.20–.31	(10,000) **
Squash, white fleshed	3–17	.04	.05–.09	200–400
yellow fleshed	3–8	.05	.08	2000–5000
Strawberries	34–60	.03	.03–.07	60–90
Sweetpotatoes	16–22	.09–.14	.05–.10	1500–7700 [d]
Tomatoes (or juice)	22–24	.06–.10	.03–.05	500–1200
Turnip greens	115–136	.09–.18	.35–.75	(10,000) **
Turnips	25–30	.05–.08	.05–.10	10–20
Watercress	40–80	.08–.15	.15–.30	800–5000
Watermelon	6–7	.03–.05	.03–.05	50–590
Wheat, entire	—	.55–.60	.10–.22	—
Wheat germ	—	1.50–2.50	.60–.80	trace
Yeast, baker's cake	—	.27–.70	.60–3.00	—
baker's dried	—	1.00–3.00	2.00–4.00	—
brewer's dried	—	5.00–9.69	2.50–5.45	—

[d] Significantly higher in yellow varieties.

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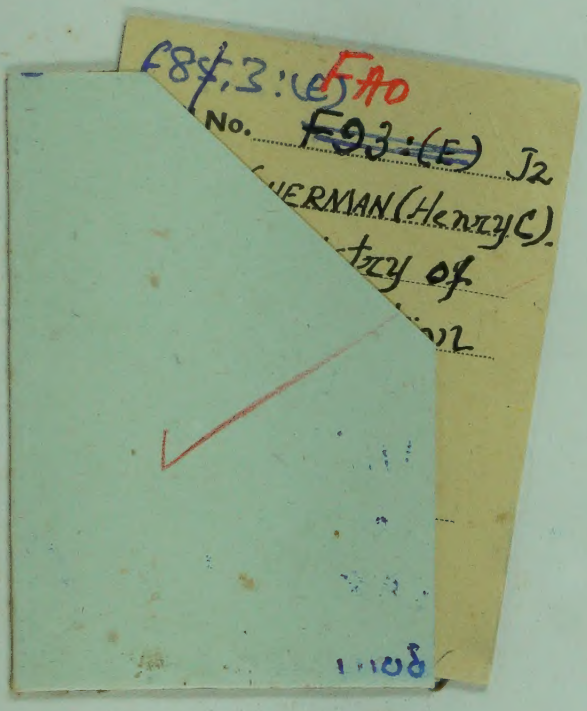
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